The Journal of Laboratory and Clinical Medicine

VOL. VIII ST. LOUIS, MO., DECEMBER, 1922 No. 3

ORIGINAL ARTICLES

THE INTERPRETATION OF BLOOD SUGAR ESTIMATIONS THAT ARE NEAR THE NORMAL*

BY HENRY J. JOHN, M.D., CLEVELAND CLINIC, CLEVELAND, OHIO

WHEN a case of glycosuria is checked by a blood sugar examination and a value of 125-155 mgm. per 100 c.c. is found, the natural tendency is to disregard this comparatively low figure and to conclude that the patient is a nondiabetic because his blood sugar is so near the normal level. The question is: In such a case how are we to determine whether the individual is normal or is in the early stages of diabetes? Perhaps this problem may be illustrated best by means of actual cases.

CASE 1.—A man, aged sixty-two, of Hungarian descent, came to the Clinic with pyonephrosis. There was no diabetes in the family and nothing of especial importance in either family or personal history. Physical examination showed a man in good physical condition with normal blood pressure. His complaint was pain in the loin which radiated down the leg.

Examination of the urine showed the presence of albumin, pus and sugar. A functional red test showed an output of phenolsulphonephthalein amounting to 40 per cent the first hour and 20 per cent the second. The Wassermann reaction was negative. Blood sugar examination showed 159 mgms. three hours after a meal and on another day 127 mgms. four hours after a meal. (Within three hours after a meal blood sugar should be normal, i. e., 120 mgms. per 100 c.c.)

Here we have a case with glycosuria which, when tested upon two different days, showed hyperglycemia also. Such a case might be dismissed as one of diabetes but, to establish the diagnosis definitely, a glucose tolerance test was made. In this particular instance 100 gms. of glucose, given on a fasting stomach, showed the following results: (See also Curve 2.)

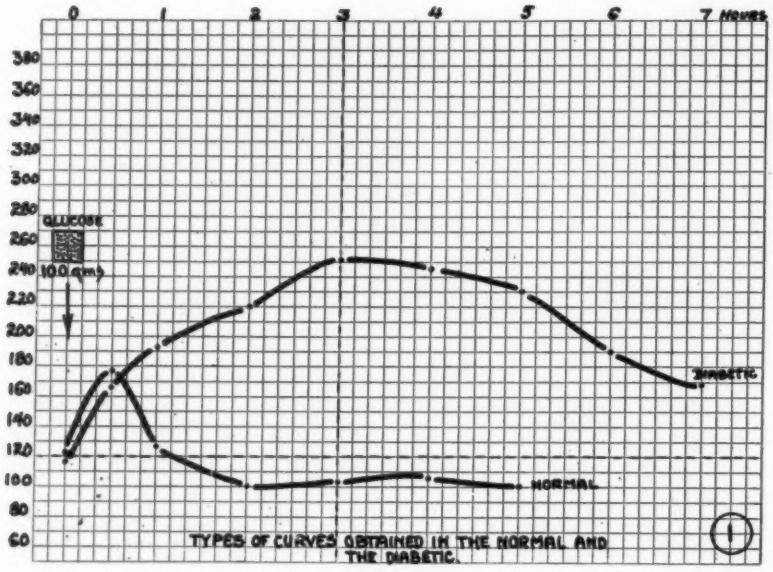
This curve, with its slow rise and equally slow fall, is distinctly diabetic.

*Received for publication, July 24, 1922.

GLUCOSE TOLERANCE ESTIMATION—CASE 1

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400				330		
300		258	272		241	
200						
150	154					133
120						
sugar in urine	trace		plus .26 gm.	2 plus 1.6 gm.	2 plus 1.2 gm.	trace .04 gm.

So the patient was given information concerning his condition and by adhering to a proper diet, which at this stage means only a slight restriction of carbohydrates and no restriction of calories, he should go through life as a mild diabetic.



EXPLANATION OF CHARTS

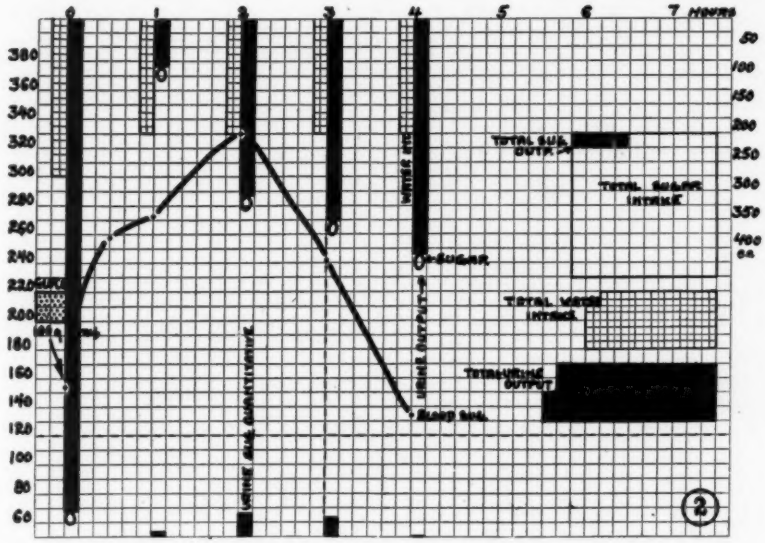
The checkered columns at the top of the charts indicate the water intake; the solid black columns at the top indicate the urine output. The total intake and output during the period of four hours or more is indicated by a like marking at the lower right corner of the chart. The circles at the lower ends of the black columns indicate the presence of sugar in the urine.

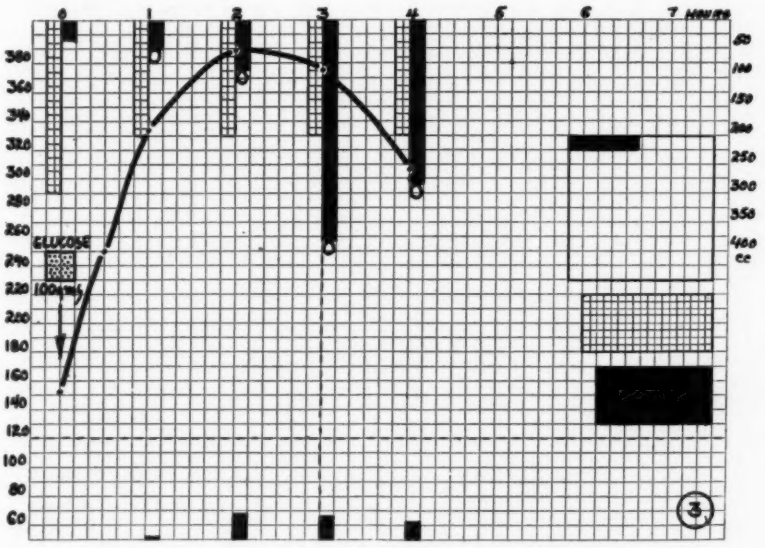
The broken horizontal line opposite "120" is the normal level of blood sugar, i. e., 120 mg./100 c.c. of blood. The broken vertical line opposite "3 hours" indicates the period within which, in normal individuals, the blood sugar content again becomes normal after the ingestion of the standard dose of glucose. The heavy curve represents the blood sugar content at the designated periods. The dots which break the glucose tolerance curve indicate the intervals at which blood was taken for sugar estimation. Each solid black column at the bottom of the charts represents the sugar content of the urine output indicated by the corresponding solid black column at the top of the chart. Each square included in these lower columns represents one gram of sugar, and the total sugar output—the sum of these squares—is indicated by the solid black portion of the large square at the right of the chart, which includes 100 squares, representing 100 gms. of glucose—the total sugar intake.

CASE 2.—A married woman, aged forty-three, reported at the Clinic complaining of loss of weight, shortness of breath and hot flashes. There was no diabetic family history and the physical examination was negative. The urine showed: acid reaction; specific

gravity of 1,022; faint trace of albumin, no sugar. Microscopic examination revealed the presence of pus. A blood sugar test, taken on a fasting stomach, showed 166 mgm. of sugar per 100 c.c. and, two days later, 151 mgm. per 100 c.c.

Here we have a middle-aged woman, going through her menopause, who has no sugar in the urine and whose fasting blood sugar is only 151-166 mgm. per 100 c.c. Is this a case of true diabetes or is it one of those excep-


0 1 2 3 4 5 6 7 HOURS
380 360 340 320 300 280 260 240 220 200 180 160 140 120 100 80 60
50 100 150 200 250 300 350 400 cc
TOTAL SUB. OUTP.
TOTAL SUGAR INTAKE
TOTAL WATER INTAKE
TOTAL URINE OUTPUT
URINE SUGAR
URINE SUB. QUANTITATIVE
URINE OUTPUT
BLOOD SUG.
2



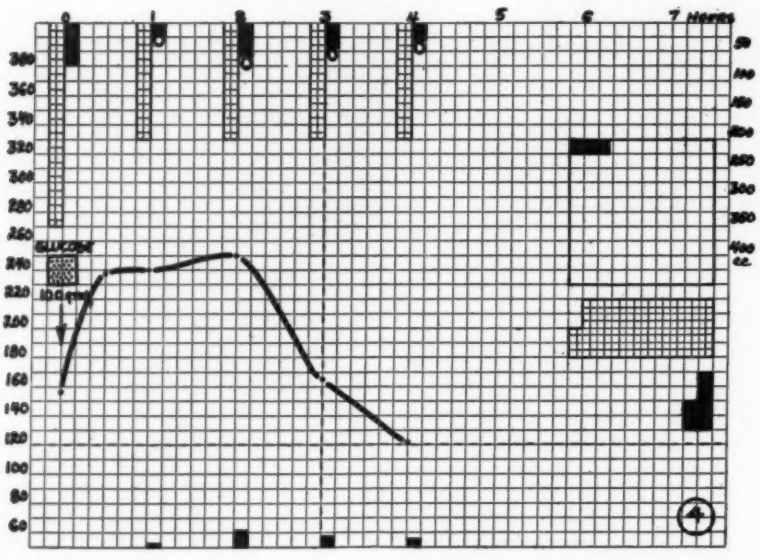
tional cases in which the blood sugar is normally above that of accepted standards? If it were a case of the latter type it would be most unfair to put the patient on a diabetic regimen. Again a glucose tolerance test in this case disclosed the true state of affairs and classified the patient as a diabetic

in spite of the fact that there was no sugar in the urine and the blood sugar was not far above normal. (See also Curve 3.)

GLUCOSE TOLERANCE ESTIMATION—CASE 2

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400			333	389	375	306
300		250				
200						
150	151					
120						
sugar in urine	neg.		plus .19 gm.	2 plus 1.87 gm.	plus 1.63 gm.	plus 1.22 gm.

CASE 3.—A physician, aged fifty-six, married, apparently in perfect physical condition, visited the Clinic in order to confirm or to disprove the diagnosis of diabetes which had been made six years previously. He had had measles and mumps in childhood, and typhoid fever



at the ages of seventeen and thirty-five. For the past six years he had been on a restricted diet and wanted to know whether this regimen was warranted in his case.

The urine showed no sugar, was free from acetone and was negative in other respects. The fasting blood sugar was 130 mgm. per 100 c.c. The Wassermann reaction was negative.

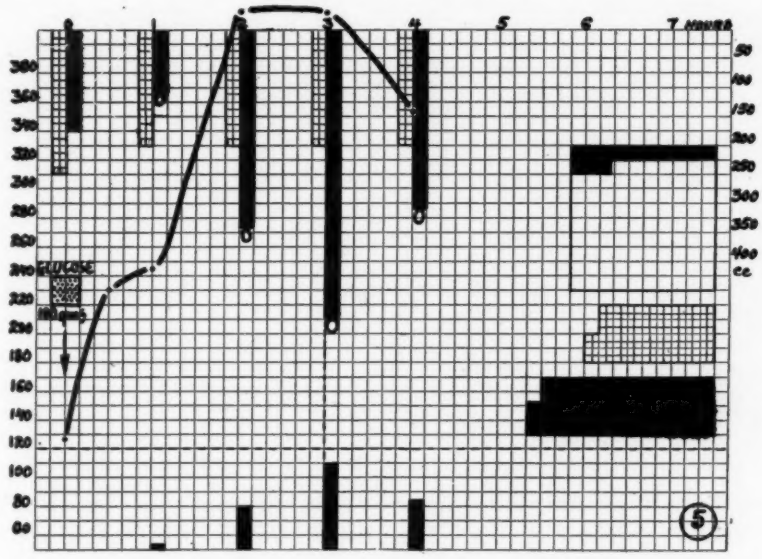
These findings shed but little light upon the justice of the diagnosis of diabetes. Until a glucose tolerance test was made any opinion would have been merely a guess. An incorrect guess, with either resulting laxity or restriction of diet, might either have entailed the breaking down of the patient's carbohydrate metabolism or have forced him to live on foods limited in amount and kind. With the information secured from the glucose tolerance test there was no hesitation about advising the patient that he was a mild diabetic and suggesting that he adhere to a diet moderately restricted as to carbohydrates. (See also Curve 4.)

GLUCOSE TOLERANCE ESTIMATION—CASE 3

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400						
300		238	240	250		
200	156				165	
150						121
120						
sugar in urine	neg.		2 plus .22 gm.	2 plus 1.1 gm.	3 plus .88 gm.	plus 0.6 gm.

CASE 4.—This patient, a clerk—languid, emaciated, pale—was the very picture of a diabetic. The patient was twenty-seven years old, had had scarlet fever at twelve, influenza four years before, several attacks of tonsillitis, and an attack of diphtheria the preceding year. A diagnosis of diabetes had been made two years before by Woodyatt, under whose care the patient had been. He was on the following diet: carbohydrates, 125 gm.; proteins, 45 gm.; total calories, 2,000.

An examination of the urine was negative for sugar and acetone. The blood sugar was 124 mg. per 100 c.c. This normal blood sugar figure in one who presented the physical signs of diabetes and who was on such a high carbohydrate and high fat diet, surprised me greatly. At the patient's request I made a glucose tolerance test which showed the following result: (See Curve 5.)



GLUCOSE TOLERANCE ESTIMATION—CASE 4

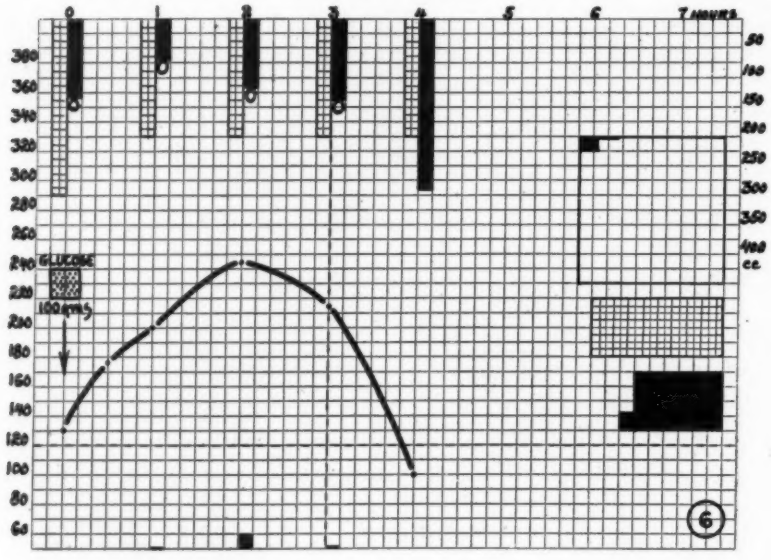
MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400				428	426	
300		230	243			352
200						
150	126					
120						
sugar in urine	neg.		plus .31 gm.	3 plus 3 gm.	3 plus 6 gm.	3 plus 3.4 gm.

With this typically diabetic blood sugar curve, which by its slow rise and slow fall showed a delay in the utilization of carbohydrates, there could

be no question regarding the diagnosis, in spite of the fact that the patient showed no glycosuria and had a normal glycemia, due to his well calculated diet. His renal permeability was high as can be seen readily from the tolerance chart so he would naturally not show any sugar in the urine unless the blood sugar were quite high. In this case, therefore, we were dealing with a diabetic under control.

CASE 5.—A Jew, aged fifty-five, married, an office worker, complained of stomach trouble and constipation. One sister had diabetes and one brother died of diabetes. There were four children, living and well. The personal history was negative. The physical examination was negative with the exception of a blood pressure of 166/90.

The urine showed a faint trace of albumin but no sugar. After an Ewald meal the gastric contents showed no free acidity and a total acidity of 26. The Wassermann reaction was negative. Fasting blood sugar was 175 mg./100 c.c.; urea, 15 mg.; uric acid, 4.1 mg.; chlorides, 625 mg. per 100 c.c. A few days after the first test, when the patient was not on a diet, the fasting blood sugar was 123 mg. per 100 c.c.



In this instance we had a middle-aged Jew, who had no complaint referable to diabetes and no symptoms of the disease. A blood sugar of 175 mg. per 100 c.c. was somewhat high, yet when this blood sugar was checked by a further observation a few days later, a normal figure of 123 mg. was obtained.

The temptation in this case would be to rule out diabetes on the grounds that there was no glycosuria present and that the blood sugar was normal. Yet, when we went a step further and made a glucose tolerance test we learned that the case was definitely diabetic. (See Curve 6.)

It is with a patient of this type that most can be accomplished for here the ravages of the disease have not yet initiated their destructive processes. The man still had a good carbohydrate tolerance and, by a slight restriction of carbohydrates, could be carried safely and comfortably through life. If treatment were postponed until the disease signs were more marked it would

GLUCOSE TOLERANCE ESTIMATION—CASE 5

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400						
300				243	214	
200		176	200			
150	130					
120						100
sugar in urine	trace		plus	2 plus	plus	neg.

still be possible to save the patient, but it would be necessary to impose a much more rigid diet which often proves an unendurable hardship.

CASE 6.—A housewife, aged fifty-one, married for thirty-five years, came to the Clinic because she had lost weight, was having hot flashes and ringing in the ears. She had had tuberculosis six years before, rheumatism twenty-five years previously and had had many attacks of gallstone colic. There were three children, living and well. The husband had diabetes but aside from that fact the family history was negative. The physical examination revealed nothing of especial importance. The blood pressure was 140/90.

Urine examination: reaction, acid; specific gravity, 1,040; faint trace of albumin; sugar 4 plus. A functional red test showed 40 per cent phenolsulphonephthalein excreted the first hour, and 14 per cent the second. The Wassermann reaction was negative. The blood sugar was 221 mg. per 100 c.c. A few days later the patient came in without glycosuria and with a fasting blood sugar of 161 mgm., which might have been considered as proof that diabetes was not present if a glucose tolerance test had not clearly told the story. (See Curve 7.)

GLUCOSE TOLERANCE ESTIMATION—CASE 6

MGM/100CC.	BEFORE	½ HR. AFTER	1 HR. AFTER	2 HR. AFTER	3 HR. AFTER	4 HR. AFTER
400						
300		280	204			
200	161			187	171	
150						129
120						
sugar in urine	neg.		3 plus .7 gm.	3 plus 2.7 gm.	3 plus 4.2 gm.	3 plus 1.1 gm.

SUMMARY

Tabulating the parallel examinations of blood and urine* in the six

*Case No.	Blood Sugar	Glycosuria
1	127	plus
2	151	neg.
3	130	neg.
4	124	neg.
5	123	neg.
6	161	neg.

cases presented above we make the following observations:

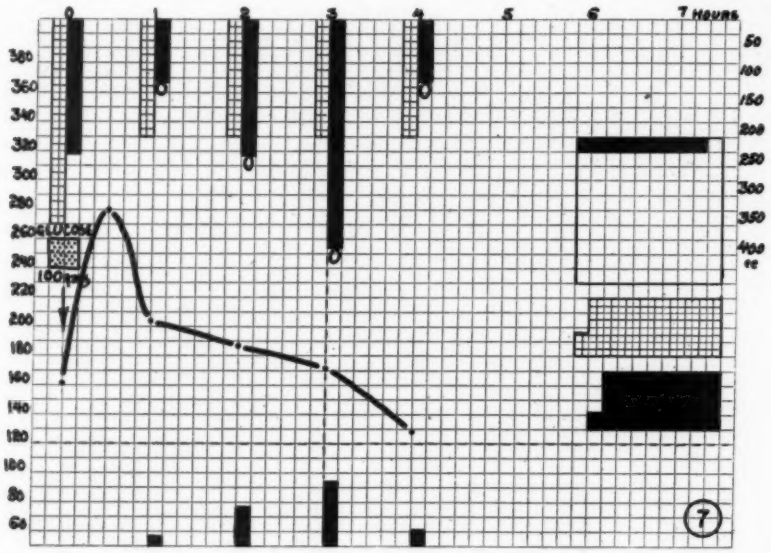
(a) Glycosuria in one case.

(b) Blood sugar near the normal in all cases.

(c) Definite diabetic curve in all cases in which a glucose tolerance test was made.

(d) Glycosuria in all cases in which the blood sugar level reached the point of renal permeability.

It is clear that we cannot regard a blood sugar level which is near the normal as *normal* unless we know under what conditions it was obtained (whether the individual is on a diet or has been eating sparingly for a time, etc.), and unless we can demonstrate by a glucose tolerance test that the slight rise is of nondiabetic origin. Borderline cases are the most difficult to diagnose and yet the most interesting and the most important. When it is seen that the borderline case has a diabetic trend a simple restriction



of carbohydrates will carry the individual through life comfortably. Usually the total calories need not be changed. On the other hand, if such a case is allowed to drift along without dietary supervision he will reach the stage of diabetes when a *marked* restriction of proteins and total calories, as well as of carbohydrates, will be necessary. In the first instance the man is kept at his work, producing and providing for his family; but if a waiting course is adopted the man is apt to become a permanent invalid and an economic burden.

OBSERVATIONS UPON THE BLOOD-FLOW IN MAN*

II. Estimation of the Blood-Flow Through the Hands in Clinical Cases

By N. B. Taylor, M.B., Ontario, Canada

IN a previous paper[1] were reported the results of a series of observations upon the blood-flow through the hands as estimated by the calorimetric method of Stewart. The calorimeter, as described in that communication, was designed in accordance with Stewart's original specifications. It consisted of a cylindrical vessel supported in a vertical position and admitted the hand through an opening in its cover. Since there was no provision against the leakage of water between the felt collar and the wrist, it was impossible

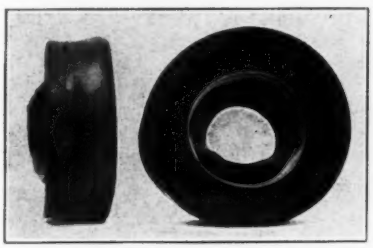


Fig. 1.

Fig. 2.

Fig. 1.—Showing side and front views of rubber sleeve.
Fig. 2.—Showing calorimeters in use in the laboratory.

to employ the instrument in any position but the vertical. This was a great disadvantage in the employment of the method for the estimation of the blood-flow of bed cases and especially of those seriously ill. It was found most difficult to place and maintain the hands in the proper position and on account of the width of the bed, since the apparatus must rest upon the floor on either side, it was impossible to observe the flow in the two hands simultaneously. The construction of the apparatus has been since modified in such a way as to allow of its being placed in the horizontal position without the leakage of water during the observation.

*From the Physiological Laboratory of the University of Toronto.

DESCRIPTION OF CALORIMETER AS MODIFIED FOR CLINICAL USE

The calorimeter is similar in general construction to that originally described, consisting of an inner vessel of copper insulated by broken cork from an outer one of galvanized iron. One end of the copper vessel is provided with a flange over which a rubber sleeve is passed and kept upon the stretch. The rubber sleeve which is an adaptation of an appliance previously employed in the treatment of war wounds[2] encircles the wrist and is especially designed, when the hand is in position, to prevent the escape of water. It consists essentially of a flange of thin rubber (Fig. 1) reinforced by

TABLE I

OBSERVATIONS UPON BLOOD-FLOW BY VERTICAL METHOD

SUBJECT	AVERAGE BLOOD-FLOW FROM A SERIES OF OBSERVATIONS UPON EACH SUBJECT GRAMS PER 100 C.C. OF TISSUE PER MINUTE	
	RIGHT HAND	LEFT HAND
F. G. B.	9.3	6.1
B(a)	8.0	8.0
C. B.	12.0	10.7
B(b)	6.2	6.6
B(c)	12.0	10.0
H. G. C.	11.7	10.8
G. D.	9.4	9.6
H. S. F.		14.1
F.	6.2	5.3
J. E.	9.4	9.8
K. M. H.		4.1
H(a)		8.0
H(b)	6.5	6.7
D. D. H.	8.2	6.7
N. R. H.	8.3	8.5
K. H.	9.5	9.2
A.	4.2	5.4
I. McC.	4.9	7.7
E. M. J.	7.1	6.8
J.	12.6	9.6
J. L.	10.5	8.5
McM.		12.8
M.		7.5
C. N.	6.2	4.8
S. U. P.	6.4	6.3
M. R.	7.5	7.4
R. S.		12.0
S(a)		3.7
S(b)	14.0	10.7
U.	8.8	5.9

Average of entire series of 30 subjects (R. H. 8.6.) Left Hand 8.1.

fingers of thicker rubber which are attached to a thick collar of the same material and from which the thinner flange also arises. The free ends of the supporting fingers are tapered and blend with the thin feather-edge of the flange. Such a construction allows of a perfectly water-tight joint to be established about the wrist and yet does not cause, by undue constriction, any interference with the return of venous blood from the hand. The apparatus may be placed horizontally upon the bed beside the patient without the fear of leakage. Fig. 2 shows the apparatus in use in the laboratory,

a hinged joint allows of its being turned into the vertical position when required.

In preparing to take an observation the water must, of course, be placed in the calorimeter while it is in the vertical position. The hand is then inserted, being passed through the sleeve to a point where the wrist is lightly gripped by the edge of the flange. At the conclusion of the observation the apparatus is turned into the vertical position while the hand is removed and the aperture is closed by a cork plug covered with rubber membrane. The instrument is then returned to the horizontal position while the readings, from which the cooling of the calorimeter is estimated, are taken.

TABLE II

OBSERVATIONS UPON BLOOD-FLOW BY HORIZONTAL METHOD

SUBJECT	AVERAGE BLOOD-FLOW FROM A SERIES OF OBSERVATIONS UPON EACH SUBJECT GRAMS PER 100 C.C. OF TISSUE PER MINUTE	
	LEFT HAND	
H. J. A.	2.5	
J. H. B.	14.0	
C. S. B.	9.3	
H. E. B.	1.4	
J. B.	8.4	
B.	6.2	
D. G. C.	7.3	
C.	4.3	
D. V. C.	3.9	
D.	7.4	
W. R. F.	11.4	
F.	4.8	
A. H.	1.5	
M. A. H.	8.0	
T. H.	1.3	
J. J.	7.1	
K.	5.3	
J. E. L.	15.4	
McL.	9.3	
M.	8.6	
J. E. M.	12.3	
J. R. N.	5.9	
F. R. P.	11.5	
A. E. R.	9.1	
M. S. R.	6.3	
D. S.	3.8	
W.	6.3	
Y.	9.8	
.....	3.2	
B. R. D.	8.1	

Average of entire series of 30 subjects—Left Hand 7.1.

A COMPARISON OF THE RESULTS OBTAINED BY THE VERTICAL AND THE HORIZONTAL METHODS

Since two new features which might have an influence upon the blood-flow, namely, the horizontal position of the limb and the encircling rubber flange, were introduced by the modified apparatus, it was considered desirable to compare the results obtained by the two methods in a large series of cases (Table I and II). It was not possible except in a few cases to com-

pare the results of the two methods when employed on the same subject. The average blood-flow of a group of individuals as estimated by the vertical method has therefore been compared with the average in another group obtained by the horizontal method. There are thirty individuals in each group and several observations over a varying period of time were taken upon each individual. The average of a series of estimations upon each subject has been taken and shown in the columns of the tables. The average of the entire group is shown at the bottom of the respective table. The general conditions of the experiment were approximately the same in the two series. Observations were taken upon the two hands in the case of the vertical method but in the employment of the horizontal method the observations were confined to the left hand. A comparison of the flow through this hand shows higher values for the vertical than for the horizontal method, being 8.1 grams per 100 c.c. tissue per minute in the former and 7.1 grams with the latter method, a difference of about 12 per cent.

SUMMARY

1. A modification of Stewart's calorimeter by which the blood-flow may be estimated while the hand is in the horizontal position is described.

2. The results obtained by the two methods are compared. The horizontal method gives values 12 per cent lower than the vertical method.

The author wishes to express his thanks to Prof. J. J. R. Macleod for his help in this research and to Miss Hearn for the preparation of the tables.

Funds for this research were furnished by the Medical Research Committee of the Department of Public Health, Ottawa.

BIBLIOGRAPHY

[1]Taylor, N. B.: Jour. Lab. and Clin. Med., 1922, vii, 439.
[2]Taylor, W. H., and Taylor, N. B.: Lancet, London, May 11, 1918.

THE RAPID PREPARATION OF POLYCHROME METHYLENE BLUE STAINS FOR FROZEN SECTIONS OF FRESH AND FIXED TISSUE*

By B. T. Terry, M.D., Nashville, Tenn.

Part I

FRESH TISSUE STAIN

MANY pathologists are called upon more or less frequently to make very quickly a diagnosis with the microscope of tissue removed surgically. For success in this work a good, reliable, rapidly acting stain is essential. Such a stain can now be made quickly and inexpensively by anyone who is willing to follow carefully a few simple directions.

Before giving my latest method of preparing stains for frozen sections I desire to state that my research on this subject grew out of a visit paid several years ago to the Mayo Clinic in Rochester, Minn. The stained frozen sections shown me there by Dr. William Carpenter MacCarty were much more beautiful and satisfactory than any I had ever seen previously and the technic of staining was wonderfully simple. This stain and technic, I understand, were introduced about 1905 by Dr. Louis B. Wilson whose article on "Staining Sections of Living Tissue, Unfixed"† contains much useful information.

The stain recommended by Dr. Wilson for unfixed tissue is a ripe, Unna's polychrome methylene blue. Formerly, according to Dr. Wilson, the best samples of this stain were obtained direct from Gruebler. During the war when this source of the stain was cut off, it was usually necessary for those using the stain to ripen it themselves. Here a real difficulty presented itself, for the ripening carried out at room temperature was a time-consuming process, requiring 6 months to a year or longer. Moreover many batches of stain spoiled in the ripening so that one was never sure of securing a satisfactory stain even after waiting a year. As this stain required only about 5 seconds for staining, and as the results with it were very beautiful, it was obvious that a more rapid and more certain method of preparing it was desirable.

After a number of experiments I learned how to shorten the time of ripening to 3 weeks and a little later I reduced this time to 6 days.‡ Further work has reduced the time of ripening to a few minutes.

In one of my early experiments I narrowly missed finding the method

*From the Department of Pathology, Vanderbilt School of Medicine, Nashville, Tennessee.

Received for publication November 11, 1922. The technic of preparing these stains and the staining results were shown at the Vanderbilt Exhibit at the Southern Medical Association meeting, Chattanooga, Tenn., November 13-16, 1922.

†Wilson, L. B.: Jour. Lab. and Clin. Med., 1915, i, 40.

‡Terry, B. T.: Jour. Am. Med. Assn., 1920, lxxiv, 1775.

I now employ. I had boiled small samples of Unna's alkaline methylene blue stain for varying lengths of time from 5 minutes to 10 hours. Every sample showed plenty of polychrome, but none of them had any strength as a stain. Many temperatures between 37° C. and 100° C. were also tried, but all failed. Months later in studying the details of my experiments I suddenly realized that I had made no tests within the first 5 minutes after boiling began. To find out what occurred in this interval I tested the development of polychrome at 15 second intervals. Much to my surprise I found that I could detect polychrome after boiling for only 15 to 30 seconds, that the polychrome was very abundant and active after 2 to 2½ minutes, but that it was weak and unsatisfactory at the end of 5 minutes. It was obvious that in the first 5 minutes of boiling the stain had ripened and aged.

Stock Solutions.—Stock solutions are convenient and save much time if several stains are to be prepared. I use two stock solutions. The first is 100 c.c. of a 1 per cent solution of methylene blue in distilled water. The medicinally pure methylene blue of Merck or Mallinckrodt, the chemically pure stain of the Will Corporation, and other good methylene blues have given excellent results in my hands. The second stock solution is 100 c.c. of a 1 per cent solution of potassium carbonate in distilled water. Merck's U. S. P. potassium carbonate I find excellent. These two stock solutions keep well and will hereafter in this paper be referred to as "Stock Solution No. 1" (1 per cent methylene blue), and "Stock Solution No. 2" (1 per cent K_2CO_3).

Preparation of Stain No. 1, or Alkaline Polychrome Methylene Blue for Fresh Tissue.—To make one ounce of fresh tissue stain, place in a clean Erlenmeyer flask of 150 to 250 c.c. capacity:

Stock solution No. 1	6 c.c.
Stock solution No. 2	6 c.c.
Distilled water	18 c.c.
	30 c.c.

Place the flask on a wire gauze supported on a tripod and heat over a Bunsen burner using a flame 6 to 7 inches high. It usually requires heating for about 2 minutes before the solution begins to boil. Boil it exactly 2½ minutes, turn out the flame and, grasping the neck of the hot flask with some convenient holder such as several thicknesses of folded paper, cool the solution quickly at the sink by pouring large quantities of tap water on the outside of the flask. When the solution is cold, pour it into a 50 c.c. graduate and with distilled water make up the stain to 30 c.c. It is desirable to filter this solution into a one ounce bottle through a little absorbent cotton moistened with distilled water and placed in the bottom of a small funnel. The stain is now ready to use. The bottle may be labelled:

"Stain No. 1
for
Fresh Tissues."

This stain gives its best results when used on frozen sections of living, unfixed tissue. As soon as the cells die the staining is unsatisfactory.

For detailed directions about the cutting of frozen sections of fresh tissue Dr. Louis B. Wilson's paper already referred to may be consulted. In my experience it is advantageous:

1. To have the tissue to be sectioned as small as is practicable and not thicker than 1 to 2 mm.

2. To embed this tissue thoroughly in ice. This is conveniently done by adding water with a small pipet to the sides of the specimen as the freezing takes place.

3. To cut the tissue as thin as possible consistent with the successful mounting of the sections subsequently. With experience and a good microtome sections 10 to 15 μ may be cut and mounted.

To Stain Sections.—When several good, thin sections have been cut they are transferred to distilled water in a small glass dish over a black background. By means of a needle inserted in a holder one section is drawn up evenly on a slide after immersing the slide in water and getting the section flat on the slide. The section is then covered with 3 to 5 drops of stain which are allowed to act from 3 to 5 seconds. Thick sections may have to be stained longer. With the point of the needle the section is fished out of the stain and transferred to distilled water in a small white porcelain dish or in a small glass dish over a white background. In this dish the section is gently shaken to wash off the excess of stain. While this washing is going on, the stain on the slide is filtered back through moist cotton into the bottle from which it came. The stain is thus used over and over again.

Mounting the Section.—The stained section in distilled water is now drawn up for the second time on a clean glass slide, is straightened out, is drained, and is at once ready for examination with the low power of the microscope. For most cases of malignancy this power suffices. If higher powers are to be employed a drop of distilled water on the under surface of a cover glass is lowered gently on the section. Any excess of water may be absorbed with blotting paper or filter paper placed at the edge of the cover.

Advantages of Stain No. 1 for Fresh Tissues.—

1. It is quickly prepared. One ounce can be made from the stock solutions in less than 6 minutes.

2. The staining of fresh tissues is very rapid, usually requiring only 3 to 5 seconds. The very short staining period makes it an exceptionally valuable stain where quick diagnoses are important.

3. The cells, nuclei, nucleoli, and mitotic figures are wonderfully sharp and clear. This makes the diagnosis of malignancy easier.

4. The sections can be examined at once mounted in distilled water.

5. As no alcohols are employed the cells are unshrunken and appear larger than they do in sections that have passed through alcohol.

6. The staining solution stains the tissues differentially, nuclei and epithelial cells are blue, connective tissue fibrils red, pink, or colorless, and smooth muscle purplish.

Disadvantages of Stain No. 1 for Fresh Tissues.—

1. For the best results this stain must be applied to living cells. If the cells are dead or fixed, they no longer stain precisely. Epithelial cells are more sensitive to deleterious influences than connective tissue and begin to stain diffusely while the connective tissue cells are still sharply stained.

2. The staining solution is not permanent. In the course of weeks or months, depending largely on the weather, the stain becomes overripe or deteriorates and fails to stain intensely. This is not a serious drawback as a new batch of stain can be prepared from stock in less than 6 minutes. As the stain is not permanent it is best to make it up in small quantities. This is the reason my directions are for only 1 ounce.

3. The sections stained are also not permanent. This may be a serious disadvantage especially in difficult cases, for it is not possible to study the tissue long before it becomes unsuitable for further study. See Part II of this paper for a stain which permits long and careful study.

4. The cutting and mounting of unfixed tissue is much more difficult than that of fixed tissue. This is due to the fact that unfixed tissue is so soft and limp that it is much harder to get it flat on the slide for examination.

Part II

FIXED TISSUE STAIN

In many instances the tissues I receive for diagnosis are already fixed and with these the fresh tissue stain does not give satisfactory results. Moreover in a large number of cases it is desirable for me to examine quickly many pieces of fixed tissue from autopsies or other sources to determine their suitableness for class sections. After working for months with the fresh tissue stain, the older methods appeared to me to be slow and unsatisfactory. It seemed highly desirable, therefore, to modify in some way the fresh tissue stain so that it would stain sharply and precisely tissues that had been fixed. If this could be done the usefulness of the stain would be greatly increased.

On studying the sections of fixed tissue stained with the fresh tissue stain I concluded that they needed clearing. I tested, therefore, a number of clearing agents and found acetic acid was the best. When dilute acetic acid (⅕ per cent to ½ per cent) was used as a quick wash after staining with Stain No. 1 tissue that had previously been well fixed in formalin, beautiful results were usually obtained. Sometimes the tissues were not washed enough and at other times they were washed too much. In either event the tissues had to be handled a second time and this consumed a little more time. It seemed desirable, therefore, so to combine the acid with the stain that the clearing and staining might take place at the same time. By so doing it was hoped that good results could be obtained more quickly and with greater constancy. Experiments soon showed that this combination could be made.

Preparation of Stain No. 2, or Acid Polychrome Methylene Blue for Fixed Tissue.—Pour into a clean Erlenmeyer flask of 500 c.c. capacity:

Stock solution No. 1	20 c.c.
Stock solution No. 2	20 c.c.
Distilled water	60 c.c.
	100 c.c.

Heat to boiling over a Bunsen burner with flame regulated to a height of about 6 to 7 inches. This preliminary heating usually requires about 5 minutes. As soon as the fluid begins to boil, note the time and boil for exactly 2½ minutes. At the end of this time turn off the gas and pour cold tap water in large quantities on the flask to cool the contents quickly to room temperature or lower. As the solution cools, the red of the polychrome appears. To the cold solution add 10 c.c. of 10 per cent acetic acid.* The solution is shaken vigorously for about 1 minute, care being taken not to let it slop out of the flask. It is then poured into a 100 c.c. graduate and with distilled water the solution is brought up to 100 c.c. if, as is usually the case, it is a little under 100 c.c. If it is slightly over 100 c.c., you may not have boiled sufficiently vigorously, but you will probably find the solution satisfactory. Filter into a 4-oz. bottle through a small funnel in the bottom of which is a little absorbent cotton moistened with distilled water. The stain is ready for use. Label it:

"Stain No. 2
for
Fixed Tissue Sections."

The total time of preparation from stock solutions is usually about 10 minutes.†

Cutting, Staining, and Mounting.—The technic of cutting, staining, and mounting fixed tissue is similar to that for fresh tissue already described, but is much easier. Fixed tissue is somewhat stiffened so that thin sections are easier to cut and to mount. Sections of well fixed tissue 10 to 15 μ thick usually require only 5 seconds to stain and are thin enough for ordinary purposes. After washing in distilled water they are remounted in distilled water on slides just as has already been described for fresh tissue. The entire time for staining, washing, and mounting a good section varies usually between 30 and 45 seconds.

Advantages of Stain No. 2 for Fixed Tissues.—

1. It is quickly made and keeps well.
2. It acts best on tissue well fixed in formalin.
3. It is precise and rapid, requiring usually only 5 seconds for staining. This makes it especially valuable for the quick diagnosis of surgical tissues.
4. It is excellent in differentiating well fixed tissues,—epithelial cells and

*One c.c. of glacial acetic acid may be used instead, but the 10 c.c. of 10 per cent acid are more agreeable to deal with and measurements are for this reason more apt to be accurate. A 1 c.c. Luer syringe graduated in hundredths for tuberculin is a convenient instrument with which to measure out accurately 1 c.c. of glacial acetic acid.

†After I had succeeded in combining acetic acid with my fresh tissue stain I found in the literature that Goodpasture, Jour. Am. Med. Assn., 1917, lxix, 998, had already described a somewhat similar method of preparing a good acid polychrome methylene blue stain for fixed tissue. His stain requires about an hour to prepare and with his stain Goodpasture recommends staining sections 1 minute. This is much longer than I find necessary either with his stain or with mine.

nuclei staining blue, connective tissue fibrils red, pink, or colorless, and smooth muscle purplish.

5. It stains nuclei, nucleoli, and mitotic figures with intensity and sharpness. This makes the diagnosis of malignancy especially easy.

6. It stains well and quickly good tissue that has been properly fixed, sectioned, and preserved subsequently for weeks or a few months in formalin. Such sections can easily be used as class material in teaching pathology and histology.

7. A good section after staining can be decolorized in dilute acetic acid, preserved in formalin, and may then be successfully restained with this stain weeks or months later.

The main disadvantage with Stain No. 2 is that the sections stained with it are not permanent. If this drawback can be efficiently overcome, the value of the stain will be tremendously increased.

Simultaneous Preparation of Stains 1 and 2.—If one desires to prepare the alkaline and acid stains at one boiling this can be done easily. Measure into a clean 500 c.c. Erlenmeyer flask:

Stock solution No. 1	25 c.c.
Stock solution No. 2	25 c.c.
Distilled water	75 c.c.
	100 c.c.

Boil exactly 2½ minutes, cool quickly under the tap, and remove 25 c.c. which are filtered through moist cotton into a bottle. Label it, "Stain No. 1 for Unfixed Tissue."

To the remainder of the stain in the flask 10 c.c. of 10 per cent acetic acid are added, the flask is shaken well, and then the contents are made up to 100 c.c. with distilled water. The solution is filtered through moist cotton into a bottle and is ready for use. Label it, "Stain No. 2 for Fixed Tissue."

Useful Points.—1. The amount of acetic acid added to the fresh tissue stain is very important. The quantity recommended is that which seems best for general purposes. If less acid is added the connective tissue fibrils stain more intensely and show more red. If more acid is used the connective tissue fibrils stain less intensely and may be almost colorless so that the epithelial cells stand out prominently by contrast.

2. The time the solution is boiled is very important in developing polychrome. Boiling for 2 to 2½ minutes seems to be best although good acid stains may be made from solutions boiled only 30 seconds or as long as 5 minutes or more. If the alkaline stain is boiled longer than 2½ minutes or if it is slowly cooled, more red is present, more crystalline precipitate forms, and the stain is less intensely active. With continued boiling the alkaline stain finally loses practically all staining power. The addition of acetic acid in proper amount restores this power by getting the active stain back in solution.

3. An emergency stain can be made in less than 5 minutes by placing in a good sized test tube:

Stock solution No. 1	2 c.c.
Stock solution No. 2	2 c.c.
Distilled water	6 c.c.
	10 c.c.

Boil this 2 to 2½ minutes, cool under the tap, and the solution is ready to use on unfixed tissue. If you wish to convert it into an acid stain, add 2 drops of glacial acetic acid, shake, and the stain is ready to use. The proportion of acid to be added to the fresh tissue stain is roughly 1 drop of glacial acetic acid to 5 c.c. of stain.

4. Artificial light, unless well corrected with daylight glass, makes the polychrome stains look redder than when examined by daylight. A good artificial light is to be preferred to poor daylight. It is more intense, more constant, and with it more detail is brought out in the sections. A strong incandescent electric light is excellent for determining the amount of polychrome in bottles of stain. Shake the bottle and in a somewhat darkened room view a thin film of the stain by transmitted yellowish artificial light reflected from a white background.

5. *Quick Diagnosis.*—When the patient is on the table under a general anesthetic and the surgeon wants a diagnosis before proceeding with the operation, the pathologist who has on hand or makes quickly both the fresh tissue stain and the fixed tissue stain, has two courses before him. Either he may freeze the unfixed tissue, section, and stain it with Stain No. 1, or he may place a small, thin, selected piece of tissue in a test tube ⅓ full of a 4 per cent formaldehyde solution (10 per cent of the commercial formalin) and may boil it for about 1 minute, cool it quickly in a large amount of cold water, freeze, section, and stain with Stain No. 2. Thin sections thus stained are usually quite adequate for diagnosing malignancy although not so beautiful* as sections of well fixed tissue. While the second method is seemingly about 2 minutes longer than the first, it may actually be shorter, for it is easy to lose 2 minutes in trying to mount limp sections of unfixed tissue. In spite, therefore, of the time taken to fix the tissue, the diagnosis by the second method can usually be made in less than 5 minutes after the tissue is received by the pathologist. Personally I prefer the second method, although at times I use both. Until you know which method you prefer it might be well to have an assistant boil up several pieces of selected tissue while the unfixed tissue is being frozen, sectioned, and stained. As soon as the microtome is not in use, the boiled sections can be cut and stained with Stain No. 2. A ready comparison of the two methods can then be made and the more satisfactory one chosen.

6. *Test Objects.*—Not all tissues are equally good as test objects. The best tissues I have found for showing the value of the polychrome stains are squamous-celled carcinomata of the cervix uteri and carcinomata of the breast, but other tissues are probably equally good.

7. *Fixation.*—The degree of fixation of the tissue affects the result

*The problem in rapid diagnosis is not the staining of the sections, but how in a few seconds to fix tissues well. On this problem I am still working.

markedly. At times some sections from a block of fixed tissue stain beautifully. Others from the same block stain poorly. Blocks of tissue are often cut too thick or are put into too little formalin to fix well. Be sure your tissue is good before you condemn the stain. Sometimes letting the sections remain for a short while in distilled water improves the staining. Necrotic tissue and imperfectly fixed tissue will stain poorly with the best of stains.

8. It is very difficult to mount without wrinkles sections of tissue on slides that are at all greasy. Thoroughly flaming both sides of slides that are supposedly clean gets rid of all traces of fat.

AN OFFER

If any one tests carefully the directions here given for preparing Stains 1 and 2 and yet fails to get good results, I will mail that person free of charge a small sample of good acid stain and a few fixed sections that should stain beautifully, provided that within three months after this article is published that person will send me his name and address and will tell me what difficulty he has had. I may be able to set him right.

ACKNOWLEDGMENTS

To my assistants and technicians past and present my cordial thanks are here expressed for the interest they have shown in my experiments and for the substantial aid they have given me.

THE ACTION OF MORPHINE, CODEINE, AND APOMORPHINE AS SHOWN BY PERFUSION OF THE MEDULLA OF THE TERRAPIN (PSEUDOMYS TROOSTI)*

By W. J. R. Heinekamp, Chicago, Ill.

WHILE morphine is characterized by its depressing action on the higher functions, McGuigan and Ross[1] have found that subarachnoid injections of morphine produce convulsions similar to those of strychnine. They believe this action is due to oxidation products of morphine, since morphine treated with nitric acid and then neutralized produces spasms more quickly when injected than morphine *per se*. They proved the site of action to be the cord. Tamura[2] confirmed the work of McGuigan and Ross and stated that the convulsant action is due to 2-nitrosomorphine which he prepared in a pure state by the action of nitrous acid on morphine. In his second article[3] he states that the stimulation in morphine poisoning is due to the oxidation products of mor-

Fig. 1.—Inhibition produced by perfusing medulla with 1-10,000 morphine sulphate.

phine. Jackson and Ewing[4] state that morphine increases the reflex excitability of the vagus center, a fact which I later[5] confirmed. The stimulating power of morphine is established, and recently Bush[6] found that morphine when perfused through the medulla of the terrapin stimulated the cardio-inhibitory center.

Codeine, like morphine, exerts a mixed action of depression and stimulation, but while it is stated to have more tetanic action, its narcotic influence is less than that of morphine. While it has been definitely proved[7, 8] that morphine is oxidized in the body, codeine apparently is not changed. However, if codeine be oxidized *in vitro*, neutralized and then injected intrathecally, it produces strong spasms, which have been proved not to be due to the added factors or to excess acid or alkalies. Codeine when injected into frogs produces spasms more quickly than does morphine.

Apomorphine is characterized by its stimulating action on the vomiting center,[9] although small doses may produce hypnosis; and in animals incapable

*From the Laboratory of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago.

Received for publication, June 30, 1922.

of vomiting a period of excitement is noticed. Solutions of apomorphine turn dark green on oxidation and apparently lose some of their potency.

In this series of experiments I have found that morphine sulphate stimulates and sensitizes the cardio-inhibitory center, later depressing it. When morphine is partially oxidized with nitric acid and then perfused, this action is more pronounced and occurs more quickly. Apomorphine hydrochloride in

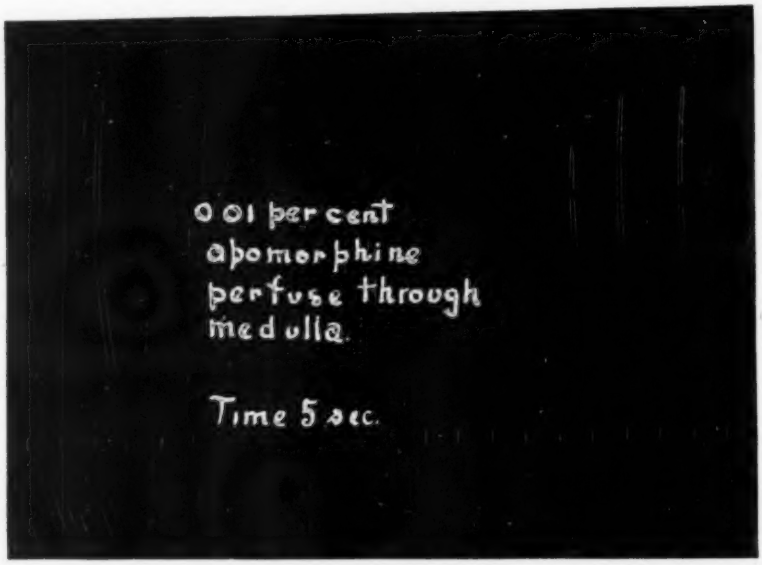


Fig. 2.—Inhibition produced by perfusing medulla with 1-10,000 apomorphine hydrochloride.

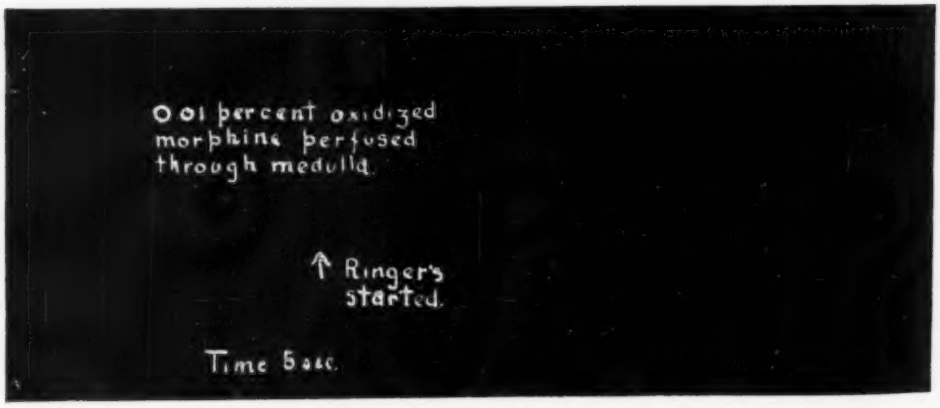


Fig. 3.—Inhibition produced by 1-10,000 oxidized morphine hydrochloride.

fresh solution first stimulates, then depresses the medulla, known stimulants failing to act after it has been used. Old solutions of a dark green color have no effect. Codeine phosphate exerts no influence *per se* when perfused through the medulla, but when oxidized with nitric acid it exerts a more powerful stimulating action on the cardio-inhibitory center than either morphine or apomorphine.

EXPERIMENTS

In the following typical experiments all the solutions used were 0.01 per cent in strength except where noted. The methods used were those employed in previous experiments:[10]

EXPERIMENT I

TIME	HEART RATE	REMARKS
—	48	Normal
11:10	48	Perfusion of medulla with codeine phosphate started.
11:30	44	Slight slowing; return to normal.
11:32	0	Vagus stimulation.
11:37	46	Perfusion of medulla with morphine sulphate started.
11:40	—	Head reflexes active.
11:42	0	Inhibition complete; return to normal with Ringer's.
12:00	36	
12:20	45	
12:25	46	After perfusion with Ringer's.
12:26	10	After perfusion of medulla with apomorphine hydrochloride.
12:30	38	After perfusion with Ringer's.
12:42	22	After perfusion of medulla with morphine sulphate.

EXPERIMENT II

TIME	HEART RATE	REMARKS
2:22	26	Normal.
2:32	0	After perfusion of medulla with apomorphine hydrochloride. Return to normal with Ringer's very slow. Slowing still evident at 2:50. Vagi active.
2:56	25	After perfusion with Ringer's.
3:15	25	After perfusion of medulla with emetine hydrochloride.
3:50	17	After perfusion of medulla with apomorphine hydrochloride.

EXPERIMENT III

TIME	HEART RATE	REMARKS
2:15	39	Normal.
2:27	39	After perfusion with morphine sulphate; reflexes.
2:35	39	Very active after perfusion with Ringer's.
2:35:10	0	After perfusion with 2-nitroso morphine; reflexes active.
2:50	39	After perfusion with Ringer's.
3:03	22	After perfusion with morphine sulphate.
3:03:20	0	After perfusion with 2-nitroso morphine.

EXPERIMENT IV

TIME	HEART RATE	REMARKS
2:20	40	Normal.
2:35	0	After perfusion of medulla with morphine sulphate; head reflexes exaggerated.

EXPERIMENT V

TIME	HEART RATE	REMARKS
10:25	28	Normal.
10:30	28	Perfusion of medulla with codeine phosphate started.
10:55	27	After perfusion of 100 c.c. of codeine phosphate.
11:20	28	Ringer's perfused since 10:56.
11:22	28	Oxidized codeine 0.005 per cent started through medulla.
11:24	14	
11:27	10	After perfusion of 5 c.c. of oxidized codeine phosphate. Ringer's solution started.
11:30:40	0	Total inhibition lasting until 11:51. Recovered.

DISCUSSION

The above experiments indicate that the various numbers of the opium group of alkaloids exert different influences on the medulla of the yellow-bellied terrapin (Pseudomys troosti).

Morphine stimulates the cardio-inhibitory center as evidened by the slowing of the heart. It also sensitizes that portion of the central nervous system exposd to it as indicated by the aggravated reflexes. This latter action is similar to that of strychnine and is in accordance with the findings of McGuigan and Ross. It is apparent from their work as well as that of Tamura and from Experiment III that the stimulating action of morphine is due to oxidation products since morphine treated with nitric acid effects these changes more rapidly and strongly than does untreated morphine.

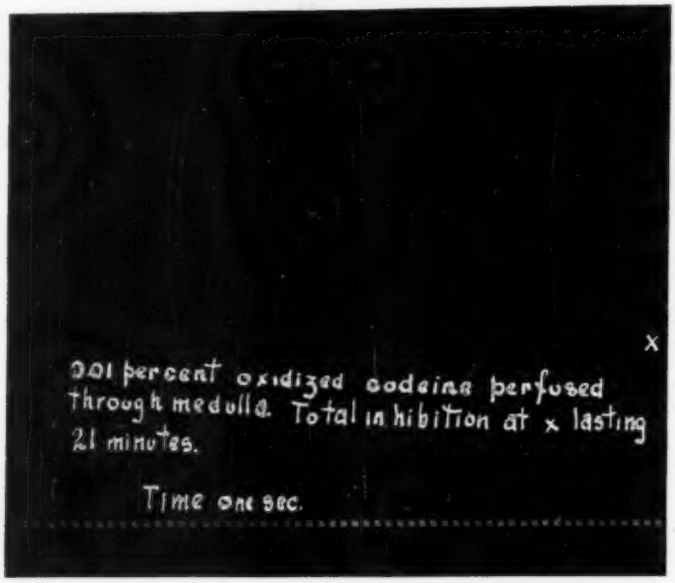


Fig. 4.—Inhibition produced by 1-20,000 oxidized codeine phosphate.

Codeine, however, does not stimulate the cardio-inhibitory center. The difference in action of these drugs may be due to the methoxy group which codeine contains, but which is absent in morphine. However, after oxidation with nitric acid, codeine exerts a more powerful and lasting stimulation than does morphine. In Experiment II, 5 c.c. of oxidized codeine produced total inhibition which lasted twenty-one minutes. It is apparent from this experiment that codeine is not oxidized in the body of the turtle as is morphine and hence it does not produce convulsions unless it is oxidized *in vitro* before it is perfused.

Apomorphine in fresh solution acts first as a powerful stimulant, but if its use is prolonged the medulla becomes paralyzed or at least irresponsive to known cardio-inhibitory stimulants. Upon standing, however, apomorphine loses this stimulating power since it effects no change in the heart rate when perfused through the medulla.

SUMMARY

Morphine sulphate when perfused through the medulla of the terrapin (Pseudomys troosti) produces inhibition of the heart due to its products of oxidation, since morphine oxidized *in vitro* with nitric acid when perfused exerts a quicker and more powerful stimulating action than morphine *per se*.

Codeine exerts no influence on the medulla, due probably to the presence of a methoxy group. However, when oxidized it, too, exerts a powerful stimulating action.

Apomorphine first stimulates, then paralyzes the medulla. Solutions which have been exposed to the air and hence oxidized are ineffective.

BIBLIOGRAPHY

[1]McGuigan and Ross: Jour. Pharm. and Exper. Therap., 1915, vii, 385.
[2]Tamura: Physiol. Abstract.
[3]Tamura: Physiol. Abstract, 1920-21, v, 195.
[4]Jackson and Ewing: Am. Jour. of Physiol., 1914, xxxiii, Proc. xxx.
[5]Heinekamp: Jour. Pharmacol. and Exper. Therap., 1919, xiv, No. 4, p. 337.
[6]Bush: Ibid., xv, No. 5, p. 173.
[7]Cloetta: Archiv. f. Exper. Path. u. Pharmacol., 1903, l, 453.
[8]Fraenkel: Archiv. f. Exper. Path. u. Pharmacol., 1919-21, lxiii, lxiv, 217.
[9]Cushny: Pharmacology, Ed. 7, p. 435.
[10]Heinekamp: Jour. Pharm. and Exper. Therap., 1922, xix, 131.

CHEMICAL CHANGES OF THE BLOOD UNDER THE INFLUENCE OF DRUGS. II. MORPHINE*

By Harry V. Atkinson and Harold N. Ets

INTRODUCTION

THIS study of the chemical changes of the blood of dogs under the influence of morphine was undertaken because of the influence of morphine on the alkali reserve and oxidations in the body. Determinations of sugar, fat, cholesterol, and creatinine were also made on the same blood sample in order to determine the relation of these changes to those above. We are also interested in the possible formation of carbohydrate from fat which Higgins and Means[1] have suggested might take place during morphine narcosis.

OXYGEN AND CARBON DIOXIDE

Filehne and Kionka[2] injected rabbits with one grain of morphine, and found a decrease in the carbon dioxide output and oxygen absorbed. They also noted an increase in the blood carbon dioxide and a decrease in the blood oxygen, and explained it as due to respiratory depression.

Loewy[3] measured the oxygen consumption of two men in normal sleep, and two in morphine sleep, and found a small drop in the oxygen consumed under morphine.

Boeck and Bauer[4] found a decreased carbon dioxide production and oxygen consumption in a dog under morphine, while in a cat in which morphine acts as an excitant, the gaseous exchange was greatly increased. A 50 per cent decrease in the metabolism of dogs and rabbits under the influence of fairly large doses of morphine was found by Fubini.[5]

Higgins and Means[1] in experiments on three men found either a slight decrease or no change in metabolism after giving one-fourth grain morphine, very little change in oxygen consumption, and a drop in the carbon dioxide elimination per minute which resulted in a drop in the respiratory quotient.

Chanutin and Lusk[6] determined the metabolism of two dogs under the influence of morphine. They gave their dogs subcutaneous injections of morphine in doses varying from 12 mgm. to 20 mgm. The first dog remained absolutely quiet in the calorimeter. Its metabolism decreased on an average of 6.2 per cent. The average respiratory quotient for this period was unchanged. A second dog manifested increased irritability with heightened reflexes after administering the drug. The basal metabolism of this dog increased by an average of 10 per cent. The average respiratory quotient increased during this period.

*From the Laboratory of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago, Illinois.

Received for publication, July 19, 1922.

ALKALI RESERVE

The increase in the alkali reserve of the blood is indicated by the experiments of Barbour, Maurer, and von Glahn[7] who observed in fasting men an increase in the alveolar carbon dioxide under the influence of the injection of small amounts of morphine; and by the findings of Underhill, Goldschmidt, and Blatherwick[8] who have, in a similar manner succeeded in producing, in normal dogs, an alkaline urine which they found persisted for a period of 24 hours or more. They found also that after the urine had regained its usual acid character, a subsequent injection of morphine might fail to elicit an alkaline urine although the hydrogen-ion concentration might be diminished appreciably. On the day of the morphine introduction they also found an increased nitrogen elimination. Hjort and Taylor[9] found that morphine sulphate administered subcutaneously to normal dogs in doses of 10 mgm. per kilo of body weight caused an increase in the alkali reserve which is maintained at a high level for many hours. They also found that morphine injected in these doses into dogs treated with chlorine—so as to develop an acidosis—temporarily prolonged the maintenance of a high alkali reserve level, but that these doses exerted an unfavorable influence upon the ultimate outcome.

Gauss,[10] in studying the effect of morphine upon the alkali reserve of the blood of man and certain animals, found that morphine administered subcutaneously in the form of sulphate to rabbits, dogs, and sheep, distinctly increased the alkali reserve of the blood plasma of these animals. Likewise there was a slight but distinct rise in the p_H of the blood plasma. Excitement in dogs and rabbits decreases the alkali reserve of the blood plasma.

Gauss also found that the normal alkali reserve of the plasma of the lamb is distinctly lower than that of sheep, but morphine increases it in both animals. Repeated short interval injections of morphine do not tend to have a greater effect than a single injection. The alkali reserve increase as a result of the injection of morphine is greater in the rabbit than in either dogs or sheep. Gauss also states that in men who were given a therapeutic or slightly toxic dose (one-fourth to one-half grain) morphine did not have a marked effect when administered subcutaneously upon the p_H or the alkali reserve of the blood plasma.

FAT

Bloor[11] found that morphine produced no change in the fat concentration of the blood of dogs during narcosis, but it did produce a considerable "after rise" during two or three days following.

SUGAR

Lepine[12] states that Coze[13] was the first to observe the increase in the reducing power of the urine and the blood of animals under the influence of morphine. Eckhard[14] is given the credit for first finding a fermentable sugar in the urine under similar conditions, but in some cases he found that the urine was levorotatory, and this to be due to a conjugated form of glycuronic

acid—with morphine. This latter fact had been previously discovered by v. Mering,[15] Mayer,[16] and others. Morphine sometimes causes pentosuria.[12]

Luzzatto[17] found that morphine causes glycosuria in rabbits and this is confirmed by Araki.[18] However, Hirsch and Reinbach,[19] state that morphine is without effect on the blood sugar concentration of rabbits.

Ross[20] and also McGuigan[21] found that morphine almost doubles the concentration of blood sugar of dogs. Auer and Kleiner[22] found a maximum increase in blood sugar in normal dogs injected with morphine and about four times as great an increase in dogs with pancreatic deficiency.

Higgins and Means[1] found no appreciable increase in the blood sugar of man under the influence of morphine.

METHODS

Healthy and well-nourished dogs were selected and placed on a liberal mixed diet. The dogs were fed late in the afternoon and a normal sample of blood drawn the next morning from the left heart through a hypodermic needle. All the influences of digestion on the composition of the blood had ceased to operate by this time. The sample of blood was too small to cause any changes due to hemorrhage. The dogs submitted without excitement in all cases. Immediately after drawing the first sample of blood, the dogs were given a hypodermic injection of one grain of morphine sulphate in divided doses. A second sample of blood was drawn two and one-half hours after the first and in some cases a third sample was taken seven hours after the first. In addition to the methods of analysis given elsewhere by the authors,[23] the oxygen capacity of the whole blood was determined according to the method of Van Slyke.[24]

EXPERIMENTAL DATA

The results obtained are given in Table I.

Upon inspecting Table I, it is seen that the carbon dioxide combining capacity of the blood plasma is increased from a normal value of 50 to 56 volume per cent, or a relative increase of 12 per cent two and one-half hours after the subcutaneous injection of morphine, and that this had further increased to 16 per cent above normal seven hours after the injection of morphine, but had returned to normal in 24 hours.

The oxygen capacity of the blood increased from 25.5 to 26.8 volume per cent, or a relative increase of 5 per cent in two and one-half hours and it had dropped 5 per cent below normal at the end of seven hours.

The normal blood sugar was found to be 0.09 per cent and this was increased to 0.14 per cent in two and one-half hours, or a relative increase of 53 per cent. This had dropped to 0.112 per cent in seven hours, but was still 23 per cent above normal.

There was only a very slight increase in the total blood fat in two and one-half hours, but a 9 per cent increase above normal existed after seven hours.

TABLE I

EFFECT OF THE SUBCUTANEOUS INJECTION OF ONE GRAIN OF MORPHINE SULPHATE ON THE CHEMICAL COMPOSITION OF THE BLOOD OF THE DOG

DOG NO.	EXPERIMENT NO.	DATE 1921-22	CO_2			O_2			SUGAR			FAT			CHOLESTEROL			LECITHIN		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
			VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	VOL. PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
IV	1	Dec. 7	43	58	46	28.3	27.2	25.3	0.093			0.83	0.86		0.178	0.159		0.20	0.21	
IV	2	Dec. 8	46	56		28.0	40.3		0.100			0.74	0.83		0.173	0.231		0.27	0.27	
VI	3	Dec. 9	44	46		26.3						0.93			0.169			0.24		
VI	4	Dec. 12	52	64	64	22.2	22.2	19.8	0.086	0.097	0.097	0.73	0.87	0.91	0.112	0.130	0.104	0.30	0.18	0.34
VIII	5	Dec. 15		54		20.3			0.068	0.120		0.85	0.95		0.125	0.139		0.47	0.45	
VIII	6	Dec. 16	54	54		27.3			0.100	0.108		0.93	0.91		0.147	0.164		0.41	0.36	
VIII	7	Dec. 19	42	53	60	18.3	21.4	18.3	0.107	0.150	0.130	1.00	1.16	1.02	0.166	0.200	0.166	0.26	0.15	0.16
VIII	8	Dec. 20	53	63	60	16.8	27.3	27.3	0.098	0.125	0.103	0.89	0.93	1.10	0.156	0.104	0.187	0.50	0.39	0.41
VIII	9	Dec. 21	53	61	58	19.3		20.8		0.187		0.78	0.85	0.80	0.093	0.096	0.09?	0.26	0.26	0.24
IX	10	Jan. 9	58	60	60	31.7	30.3	33.3	0.077	0.140	0.120	0.91	0.97	1.05	0.140	0.150	0.120			
IX	11	Jan. 11	55	55		30.8	34.8		0.072	0.118		1.00	1.00		0.170	0.160				
IX	12	Jan. 13	55			30.4	33.4		0.105	0.200		0.94	0.93		0.167	0.131				
IX	13	Jan. 16	47	50		30.4	30.2		0.970	0.143		0.95	1.05							
AVERAGES			50	56	58	25.5	26.8	24.1	0.091	0.139	0.112	0.88	0.94	0.98	0.15	0.15	0.13	0.32	0.29	0.29
CHANGE, PER CENT				+12.0	+16.0		+5.0	−5.0		+53.0	+23.0		+6.0	+10.0			−13.0		−9.0	−9.0

Column A contains the readings taken for a normal dog just before injecting morphine; Column B, two and one-half hours after injecting morphine; Column C, seven hours after injecting morphine.

The cholesterol first remained constant and then dropped 13 per cent below normal in seven hours.

The lecithin showed a decrease from the start and remained about constant at 9 per cent below normal.

Based on a limited number of results, not given in the table, the creatinine increased from 2.5 milligrams per 100 c.c. of whole blood to 3.2 milligrams or relatively 28 per cent in two and one-half hours, but had dropped from this high level to 2.9 milligrams in seven hours or 15 per cent above normal.

DISCUSSION

The fact that the carbon dioxide capacity of the blood plasma at the end of two and one-half hours was relatively increased 7 per cent more than the oxygen capacity, is of interest in connection with its effect on the oxidations taking place in the body. This is even more striking at the end of seven hours since by this time the carbon dioxide capacity had increased further and the oxygen capacity had dropped below normal, thus giving a relative difference of 21 per cent between these two. This would in all probability materially retard oxidations in the body.

In this connection the findings of Higgins and Means[1] are very significant. They found a fall from .75 to .70 in the respiratory quotients of human beings under the influence of morphine. These lowered respiratory quotients have been explained by these authors as being due to a change in the character of the metabolism, such as incomplete combustions, resulting in the formation of acetone bodies from fat or by changing of the fat to sugar. However, the formation of acetone bodies should cause the acidity of the blood, exclusive of carbon dioxide, to increase, which Higgins and Means[1] found not to be the case. This is confirmed in our experiments by the fact that the carbon dioxide capacity of the blood plasma is increased, indicating that there is no acidosis. The possible formation of sugar from fats would result in a very low respiratory quotient since an oxygen poor substance is being converted into an oxygen rich substance; this transformation has not yet been proved in dogs and human beings, but probably takes place in hibernating animals. Pembrey[25] also found a considerable fall in the respiratory quotients of dormice and marmots during their winter sleep. The respiratory quotients of his animals were much below that given by the oxidation of any one of the foodstuffs, fat, carbohydrate, or protein. In the experiments of Pembrey the body temperature of his animals dropped from 30° C. to 12° C., and this must of necessity diminish the oxidation processes taking place in his animals and would in all probability cause these to stop short of carbon dioxide formation, and this intermediate stage could be carbohydrate formation.

Chanutin and Lusk[6] found no decrease in the respiratory quotients of dogs under the influence of about three grains of morphine but they also record that their dogs vomited and defecated. We gave one grain of morphine sulphate in divided doses in order to prevent vomiting and rejected

all experiments in which there was vomiting because we found these results were very irregular. Usually there was no increase in the alkali reserve. We ascribed their results to vomiting and not to morphine. However, the changes in the composition of the blood under the influence of vomiting should be investigated more fully because of its effect on the alkali reserve of the blood which in turn may affect the metabolism.

The sugar increased 53 per cent during the first two and one-half hours, but was only 23 per cent above normal at the end of seven hours. Could this have been formed from fat? It is noted that the total blood fat showed very little change at the end of the period, but at the end of seven hours, it had increased 9 per cent. It is, of course, impossible to say whether the fat used to form glucose, if it be formed, would come from blood fat, in which case the blood fat should be decreased, or whether it would come from fatty depots of the body, or both. One would expect the maximum production of sugar from fat to occur at the time of the maximum change in the relation between the carbon dioxide capacity and the oxygen capacity.

It is noticeable that the cholesterols and lecithins decreased during the experiments.

SUMMARY

Morphine increases the carbon dioxide combining power of the plasma and the following constituents: Sugar, creatinine, and total fat. The lecithins, cholesterol, and oxygen combining power are decreased under the same conditions.

BIBLIOGRAPHY

[1]Higgins, H. L., and Means, H. M.: Jour. Pharmacol. and Exper. Therap., 1915, vii, 1.
[2]Filehne, W., and Kionka, H.: Arch. f. d. ges. Physiol., 1895, lxii, 201.
[3]Loewy, A.: Berl. klin. Wchnschr., 1891, xxviii, 434.
[4]Boeck, H., and Bauer, J.: Ztschr. f. Biol., 1874, x, 336.
[5]Fubini: Moleschotts Untersuchungen, 1881, xii, 563. Taken from Higgins and Means.
[6]Chanutin, A., and Lusk, G.: Jour. Pharmacol. and Exper. Therap., 1922, xix, 359.
[7]Barbour, H. G., Maurer, L. L., and von Glahn, W. G.: Jour. Pharmacol. and Exper. Therap., 1916, xiii, 124.
[8]Underhill, F. P., Blatherwick, N. R., and Goldschmidt, S.: Proc. Soc. Exper. Biol. and Med., 1916-17, xiv, 83.
[9]Hjort, A. M., and Taylor, F. A.: Jour. Pharmacol. and Exper. Therap., 1919, xiii, 407.
[10]Gauss, H.: Jour. Pharmacol. and Exper. Therap., 1921, xvi, 475.
[11]Bloor, W. R.: Jour. Biol. Chem., 1914, xix, 1.
[12]Lepine, R.: Le diabete sucre, Paris, 1909, 311.
[13]Coze, C. R.: de l' acad. des. Science, 1857, xlv, 354.
[14]Eckhard: Beitr. z. Anat. u. Physiol., 1877, viii, 77.
[15]v. Mering: Berl. klin. Wchnschr., 1874, p. 246.
[16]Mayer, P.: Berl. klin. Wchnschr., 1899, pp. 591 and 617.
[17]Luzzatto, R.: Arch. f. Exper. Path. u. Pharmakol., 1905, lii, 95.
[18]Araki, T.: Ztschr. f. physiol. Chem., 1891, xv, 546.
[19]Hirsch, E., and Reinbach, H.: Ztschr. f. physiol. Chem., 1913, lxxxvii, 122
[20]Ross, E. L.: Jour. Biol. Chem., 1918, xxxiv, 335.
[21]McGuigan, H.: Jour. Lab. and Clin. Med., 1918, iii, 2.
[22]Auer, J., and Kleiner, I. S.: Jour. Exper. Med., 1918, xxvii, 49.
[23]Atkinson, H. V., and Ets, H. N.: Jour. Biol. Chem., 1922, lxx, 5.
[24]Van Slyke, D. D.: Jour. Biol. Chem., 1918, xxxiii, 127.
[25]Pembrey, M. S.: (a) Jour. Physiol., 1901, xxvii, 66. (b) Jour. Physiol., 1903, xxix, 195.

PATHOGENICITY OF ORGANISMS COMMONLY REGARDED AS SAPROPHYTES*

By Ellis Kellert, M.D., Albany, N. Y.

AMONG the bacteria usually classified as saprophytes are certain forms often associated with disease processes. These organisms are most frequently found in the body as harmless commensals and when isolated from inflammatory lesions the tendency is to regard them as contaminants and to make further search for known pathogenic bacteria. In many instances repeated examination reveals only the same organisms and one is forced to the conclusion that they are the etiologic factors concerned.

Even so indifferent an organism as the B. subtilis may, if accounts be true, present marked pathogenic characteristics. As long ago as 1897, Charrin and deNittis[1] found that the hay bacillus attained considerable virulence by passage through animals. An extremely unusual case is that described by Lindberg[2] in which the B. subtilis was found as the cause of acute meningitis. Ophthalmia due to the same organism has been described by Silberschmidt,[3] and Spiegelberg[4] and others[5, 6, 7] have observed gastrointestinal disturbances apparently due to the hay bacillus.

In a case of meningitis secondary to otitis media in an infant, Greenthal[8] isolated the B. acidi lactici from the cerebrospinal fluid. Other saprophytic bacteria concerned in the causation of meningitis are M. crassus, M. flavis and M. catarrhalis as reported by Flexner[9] and more recently by Arce.[10] Schultz[11] describes in detail an instance of septicemia and acute vegetative endocarditis due to the M. pharyngitidis siccae which is usually saprophytic in the nasopharynx. This organism was isolated twice from the blood during life and at necropsy was obtained from the heart-valves and spleen. Teacher and Kennedy[12] cite a case of purulent meningitis due to the M. flavus.

This brief but incomplete review of the literature and our own experience indicate that saprophytic bacteria may not infrequently be responsible for severe and even fatal infection. In most laboratories it is the correct practice to view such organisms as contaminants or secondary invaders but their possible primary pathogenic characteristics must be borne in mind. In our own work we have occasionally obtained saprophytic bacteria in pure culture from such lesions as abscesses, meningitis, pleuritis, cholecystitis, etc. In a few instances the findings were so unusual as to warrant publication and so are recorded herewith.

MICROCOCCUS CATARRHALIS MENINGITIS

This patient, a boy six months old, became acutely ill with fever and "apparent distress in the head." There was no nausea or vomiting, no

*From the Bender Hygienic Laboratory, Albany, N. Y.
Received for publication, July 27, 1922.

pupillary changes. Physical examination was negative, but a lumbar puncture was performed and 15 cc. of slightly turbid fluid removed. The cell count showed 25 per cubic millimeter, globulin 3+ and smears, an increased number of cells, chiefly polynuclears and gram-negative, biscuit-shaped diplococci, extracellular and intracellular. Cultures yielded numerous colonies of similar cocci. These grew readily on ordinary media with the morphology and characteristic sugar reactions of M. catarrhalis. The child improved so rapidly that a second lumbar puncture was not performed. Ten months later the patient is reported as well.

STREPTOCOCCUS EQUINUS

The S. equinus frequently obtained in culture from the exposed surfaces of the body has been isolated by us from an infected hydrocele. The patient, a farmer sixty-four years of age, presented a large painful swelling in the

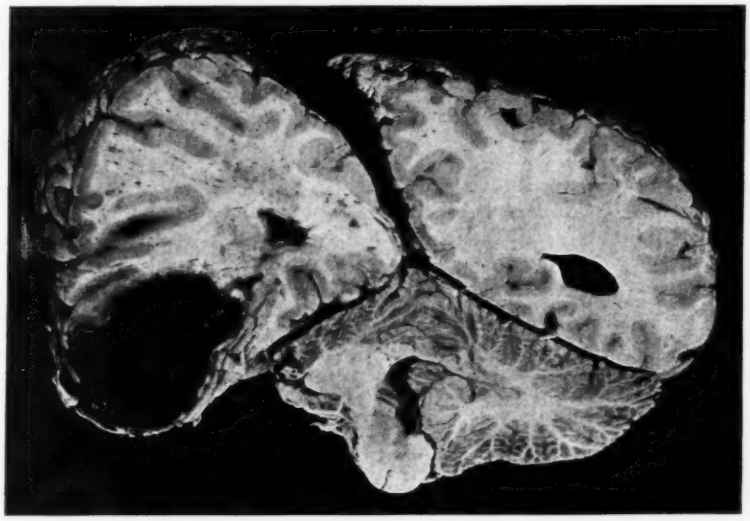


Fig. 1.—Cerebral abscess due to the B. proteus vulgaris.

right scrotal region of four years' duration. When removed, the testicle and epididymis appeared normal The tunica vaginalis was greatly thickened and filled with thick creamy pus. Histologic examination of the tunica vaginalis shows the surface covered with a layer of granulation tissue markedly infiltrated with polynuclear, mononuclear leucocytes and eosinophiles. Newly formed blood vessels are present in large numbers. Many of the larger mononuclear cells resembling endothelial leucocytes are filled with old blood pigment. The surface of this granulation tissue is covered with a layer of fibrin and polynuclear leucocytes.

Smears and cultures from the pus yielded a streptococcus finally identified as the S. equinis. The organism grew readily on blood-agar, producing no change. In semisolid media there was fermentation of salicin but no fermentation of lactose or mannite.

In this connection it is of interest to refer to an instance[13] in which a

closely related organism, the Streptococcus fecalis, was isolated from the blood of a patient suffering from acute appendicitis.

BACILLUS FECALIS-ALKALIGENES

This organism, a normal inhabitant of the intestinal tract has frequently been suspected of causing profound infection. Now and then the B. fecalis alkaligenes is encountered as the cause of prolonged febrile conditions which in certain instances resemble typhoid fever.[14] In a few of these cases[15] the lesions of typhoid fever are present; in others no such pathologic changes are found.

In our case, the patient, a man forty-three years of age entered the hospital complaining of intestinal hemorrhage. No other historical facts were ascertained. On examination, jaundice was noted, the heart and lungs were found normal, spleen slightly enlarged and the liver distinctly enlarged. Tympanites present. Leucocyte count 9,000 and one Widal reaction reported positive. The Widal reaction was not repeated and no blood culture was taken.

The necropsy revealed acute suppurative peritonitis involving the right half of the peritoneal cavity, acute pyelitis, multiple abscesses of liver. The appendix showed no evidence of primary inflammation. No hyperplasia of the lymph follicles or Peyer's patches and no ulcerations were found. The histologic examination revealed an unusually large number of plasma and endothelial cells in the mucosa. The abscesses within the liver consisted of masses of polynuclear leucocytes in various stages of necrosis, a few large mononuclear cells and numerous endothelial leucocytes at the periphery. In the center of the abscesses were seen colonies of bacteria, chiefly short slender rods. No cocci identified. Smears and cultures from these abscesses yielded the B. fecalis-alkaligenes in large numbers. No other bacteria found. The peritoneal exudate, however, showed mixed infection, streptococci present.

BACILLUS AEROGENES

The B. aerogenes, also a normal inhabitant of the intestine, is frequently found in water and milk. Occasionally it occurs as a secondary invader in infections of the genitourinary tract, but is rarely encountered elsewhere. In the case herein cited, the B. aerogenes was isolated in pure culture from the pus derived from an acute cholecystitis.

The patient, a married woman, thirty-five years of age, with negative past and family history, developed jaundice, temperature 103° F., pulse 130, respirations 30 and pain and tumor mass in the upper right abdominal quadrant. The blood showed a leucocytosis of 16,000 and a polynucleosis of 80 per cent. At operation a greatly enlarged and inflamed gall bladder was found surrounded by many adhesions. The gall bladder contained "a pint of pus" which when received was streaked with blood and strongly mucoid in character.

Smears from the pus showed numerous pus cells and many gram-negative short bacilli surrounded by faint negative capsule zones. No true cap-

sules could be demonstrated, however. The pus when plated on blood-agar yielded colonies of one type. These were large, round, moist and mucoid, but did not have the strongly mucoid and confluent character of B. mucosis. It grew readily on ordinary media, giving the reactions of organisms classified as B. aerogenes and is not, we feel certain, the B. mucosus. Particularly noteworthy were the active acidification and coagulation of milk and gas formation. When injected into mice, guinea-pigs and rabbits, no marked pathologic changes were induced.

BACILLUS PROTEUS

The B. proteus vulgaris is well known as a cause of intestinal infection, being responsible at times for summer diarrhea although TenBroeck and Norbury[16] after extensive investigation arrived at the conclusion that it played a secondary part in infectious diarrhea. When found in lesions outside the intestinal tract, the B. proteus is usually associated with other organisms and is regarded as a secondary invader. In the case about to be related, it was isolated from a cerebral abscess and not associated with other bacteria.

The patient, a man aged twenty-eight years, with a previous history of chronic otitis media, entered the hospital in a partially comatose condition, with signs of cerebral disturbance. A diagnosis of abscess was made.

A radical mastoid and a decompression operation was performed on the left side and the brain exposed. The left temporal lobe was found to bulge considerably and when incised about one ounce of pus was obtained. The patient subsequently died and at the necropsy an abscess 2 × 4 cm. was found situated in the posterior portion of the left temporal lobe. Considerable clotted blood was present and replaced the pus previously removed. Cultures from the abscess and from the pus removed at operation yielded B. proteus in pure culture. No other organisms were found in smears from the abscess.

On histologic examination the abscess cavity is found lined by a thick layer of granulation tissue in which lymphocytes, plasma cells, and endothelial leucocytes are very abundant. A few newly formed capillaries are present and there is considerable interstitial hemorrhage. Within this layer are masses of polynuclear leucocytes and other cells in various stages of disintegration. The abscess is surrounded by a very distinct layer of fibrous tissue.

These few instances lead us to conclude that under favorable conditions many micro-organisms classified as saprophytes may induce in man profound lesions and even death. Bacteria classified as nonpathogenic when found in association with acute infectious processes are not necessarily secondary invaders or contaminants.

REFERENCES

[1]Charrin and deNittis: Compt. rend. Soc. de biol., 1897, p. 49.
[2]Lindberg, G.: Hygeia, 1916, lxxviii, No. 15.
[3]Silberschmidt: Ann. Inst. Past., 1903, xvii, 268.
[4]Spiegelberg: Jahrb. f. Kinderheilk., 1899, xlix, 194.
[5]Flugge: Ztschr. f. Hyg., 1894, xvii, 272.
[6]Ardoin: Thèse de Paris, 1898, p. 78.
[7]Vincent: Intestinal Toxemia in Infants, 1911.
(References 3-7 are taken from Kendall's Bacteriology.)

[8]Greenthal: Am. Jour. Dis. of Children, 1921, ii, 203.
[9]Flexner: Studies from the Rockefeller Institute for Med. Res., 1917.
[10]Arce: Anales de la Fac. de Med. de Lima, 4, 21, p. 159.
[11]Schultz, P. T.: Jour. Am. Med. Assn., Nov. 23, 1918.
[12]Teacher and Kennedy: Lancet, London, Sept 28, 1918.
[13]Brown and Cade: Lancet, London, Jan. 15, 1921, p. 118.
[14]Rochaux and Blanchard: Annales de Med., Paris 8, p. 3.
[15]Ravenel: Jour. Am. Med. Assn., lxxvi, 720.
[16]TenBroeck and Norbury: Boston Med. and Surg. Jour., clxiii, No. 8, p. 280.

ACTINOMYCOSIS OF THE GASTROINTESTINAL TRACT: A STUDY OF FOURTEEN CASES*

By James Chester Brogden, M.D., Rochester, Minn.

THE diagnosis of actinomycosis of the intestinal tract is rarely made until late after sinus formation and extensive involvement of the intestines, liver, or other organs has taken place, and after surgical excision of the diseased area is impossible. Therefore, any mass which occurs in the lower part of the abdomen in the region of the ileocecal juncture, particularly one associated with a persistently discharging sinus, should be regarded with suspicion. Repeated examination of the discharge, pathologic examination of tissue from the mass or from the walls of the sinus, marked loss of weight and strength, pronounced anemia, flexion of the thigh without disease of the vertebral column or hip joint, and marked constipation rarely fail to establish the correct diagnosis.

DIFFERENTIAL DIAGNOSIS

In the differentiation of tumors of the intestine and actinomycosis, carcinoma should be particularly considered. Its course is more chronic, it increases in size very slowly, anemia is not as pronounced, diarrhea with blood in the stools is more often observed, the mass is generally movable, is not tender, and shows little or no tendency to invade the abdominal wall or to form sinuses. Early diagnosis of both conditions can be made only by operation and pathologic examination of the tissue.

The differentiation of tuberculosis of the intestine and actinomycosis is also difficult until the later stages. Tuberculosis often forms a chronic, slow-growing tumor in the intestine, usually associated with pulmonary tuberculosis; diarrhea is common but there may be constipation. Sinuses do not form; the growth is slower, the mass generally movable, and tuberculosis bacilli can sometimes be found in the stools. The adjacent organs, particularly the liver, are not affected as in actinomycosis. Tuberculosis, carcinoma, and actinomycosis of the intestines all show a filling defect on roentgen ray examination of the colon. In all there is anemia, loss of weight and strength, and in tuberculosis and actinomycosis, elevation of temperature after secondary infection takes place.

Appendiceal abscess is often the beginning of actinomycosis infection,

*From the Mayo Clinic, Rochester, Minn.

but most observers believe that the appendicitis is caused by the pyogenic bacteria and that this break in the mucosa allows the ray-fungi to penetrate the connective tissue in the wall of the intestine where they begin to grow. *Actinomyces bovis* has been found in the lumen of the appendix without inflammatory changes in the mucosa. Tubal abscess and localized peritonitis are more acute; they cause marked elevation of temperature. Pain is more intense, and constitutional reaction is more or less marked.

In gumma of the liver there is a history of infection; the Wassermann reaction is generally positive and other syphilitic lesions in the body establish the differentiation. In actinomycosis the abscess of the liver is always secondary to a primary focus in the intestine, or, rarely in the thorax. In amebic abscess of the liver there is a history of diarrhea and the ameba can often be found in the stools. There is no tumor in the lower abdomen and no discharging sinus. At operation the ameba can often be found in the purulent material in the abscess, while in actinomycosis the ray-fungi are easily recognized.

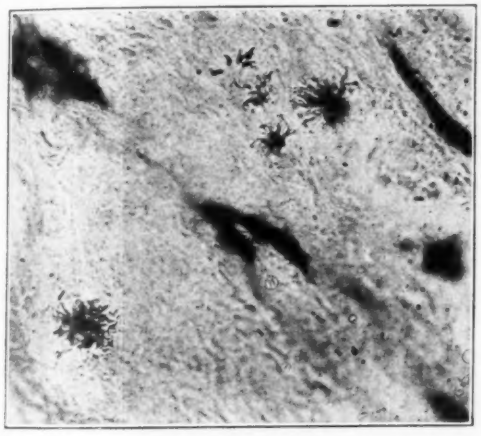


Fig. 1.

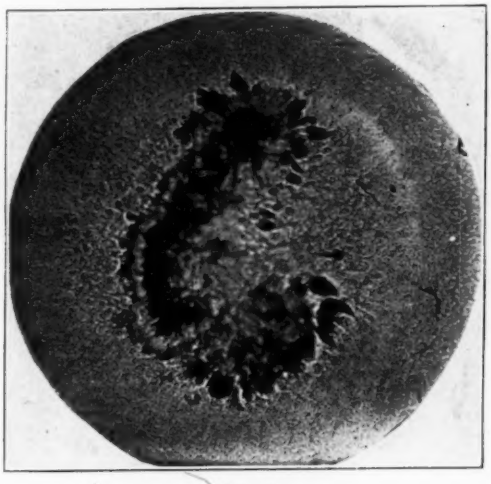


Fig. 2.

Fig. 1 (Case A210659).—*Actinomyces bovis* in the tissues (x 200).

Fig. 2 (Case A210659).—*Actinomyces bovis* showing mycelia and clubs. Extensive round-cell infiltration and necrosis are around the organisms.

PROGNOSIS

The prognosis depends on the degree, nature, and extent of the lesions, the virulence of the organism, the resistance of the individual, and the time treatment is instituted. If an early diagnosis is made so that complete resection of the intestine and the diseased area can be accomplished, and vigorous iodid, and roentgen ray and radium treatments are given, the prognosis is fairly good. In the later stages, with involvement of the other organs in the abdomen and sinus formation, the possibility of a cure is always doubtful; most observers place the mortality from intestinal actinomycosis at 60 to 70 per cent.

In the series of fourteen patients studied in the Mayo Clinic, no report

of present condition could be obtained of three; one has been treated for only four weeks. Six of the remaining ten are dead, with an average length of life after onset of the disease of three years. One patient was operated on and had no symptoms of recurrence four years afterwards. One patient is apparently cured after four years of medical treatment, and two are markedly improved after taking iodides, and roentgen ray and radium treatments.

TREATMENT

The treatment may be surgical, medical, or hygienic. Surgical treatment is usually successful only when the disease is still localized and foci of infection can be completely removed. After sinus formation and secondary involvement of adjacent structures, the sinuses should be laid open, necrotic tissue curetted out, the wound packed with iodoform gauze or swabbed with strong tincture of iodin, and later irrigated with antiseptic solution, such as 1 per cent copper sulphate or 4 per cent formalin.

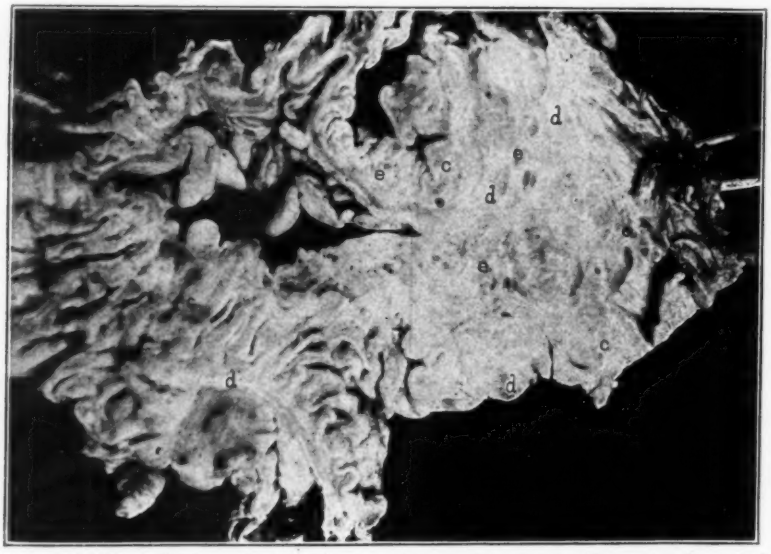


Fig. 3 (Case A210659).—Section of resected mass showing (*a*) cecum, (*d*) areas of marked fibrous tissue formation, and (*e*) areas of central necrosis.

The drugs generally used in the treatment are the iodides and copper sulphate. Potassium iodide is given by mouth in rapidly increasing doses of from 25 to 60 grains to 200 grains three times a day. Sodium iodide, 10 per cent solution, is given intravenously at first in doses of 20 to 30 c.c. and rapidly increasing to 100 c.c. a day. Copper sulphate, 0.5 grain three times a day is being used by some authorities, who report excellent results in both actinomycosis and blastomycosis. Yeast by mouth daily, with salvarsan 0.2 to 0.6 gm. every six to eight days, has been tried, but without noticeably good results. Roentgen ray and radium, continued over a long period combined with the iodides, are giving the best results and are generally successful in the early stages of the disease. Tonics and stomachics are given.

Fresh air, sunshine, moderate exercise, an easily digested, nourishing

diet, or even forced feeding, are absolutely essential, especially in the later stages in which cachexia, and loss of weight and strength are marked.

However, a review of the literature[1-13] on the subject and study of this series shows that prognosis is unfavorable in all cases of actinomycosis of the intestinal tract. The mortality is 60 to 70 per cent. Treatment is successful only when diagnosis is early before sinus formation and involvement of adjacent organs, so that the primary foci can be entirely removed and later roentgen ray, radium, and the iodide treatments given. A study of fourteen cases of actinomycosis of the gastrointestinal tract in the Mayo Clinic since 1909 seems to warrant conclusions as follows:

1. Actinomycosis of the gastrointestinal tract is not so rare as has been generally believed; thirteen of the fourteen Mayo Clinic cases occurred in the last four years.

Fig. 4 (Case A278991).—Section of resected mass showing (*a*) cecum, (*b*) ileum, and (*d*) dense fibrous tissue, and (*e*) areas of necrosis.

2. The acute form is more common; it usually begins as a typical attack of acute appendicitis. There may be one severe or several mild attacks. Six of the patients in the series gave histories of operation when a ruptured, gangrenous appendix had been removed immediately preceding the appearance of the mass. The chronic form is insidious in onset with very slight indefinite pain in the lower abdomen. There may be practically no pain, the mass being the first sign of the disease.

3. In both forms marked loss of weight and strength, pronounced anemia, induration and a bluish red discoloration of the skin over the mass and around the sinuses, and pain down the right thigh with flexion of the thigh on the abdomen are characteristic symptoms. Diarrhea is not a constant symptom of the disease. Constipation was the rule in the fourteen cases.

4. The anaerobic organism, *Actinomyces Bovis*, which possibly normally

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A30603 10/18/09	M, 51	Druggist, Iowa.	For five years attacks of dull pain in right side of abdomen lasting one to three days. July, 1908, severe pain for twelve hours and mass in upper abdomen, pain and tenderness increasing.	Tender mass in right upper quadrant of abdomen, increased liver dullness.	Intestinal actinomycosis with sinus formation, actinomycetes found in pus in January, 1916.	At operation, October 30, 1909, abscess in gall bladder region drained, 180 c.c. pus and much necrotic tissue. Second operation, December 12, 1909, pelvic abscess drained through right lower quadrant. Potassium iodide by mouth. January, 1916, another abscess drained in left lower quadrant.	Patient died, February, 1917, from extension to liver and thorax.
A148995 12/31/15	M, 40	Plumber, Ohio.	March, 1916, dull pain in left lower quadrant lasting five days, similar attack in July lasted three weeks, October 6, severe pain in right lower quadrant made operation necessary.	Large, hard, indurated mass in right lower quadrant with two sinuses discharging yellowish pus. Hemoglobin 41 per cent, leucocytes, 13,000.	Intestinal actinomycosis with sinus formation, pus showed actinomycetes.	Sodium iodide intravenously, and potassium iodide by mouth.	In 1917, the patient reported that no mass could be felt, he had gained in weight.
A207771 7/12/17	M, 35	Farm laborer, often worked with cattle with lumpy jaw, Montana.	For fifteen years recurrent attacks of dull pain in right lower quadrant. Since appendectomy, June, 1917, sinus in old wound drained yellowish pus.	Sinus draining yellow pus and hard, irregular mass in right lower quadrant.	Intestinal actinomycosis involving abdominal wall with sinus formation, actinomycetes from tissue from abdominal wall and pus.	Massive doses of potassium iodide by mouth and roentgen ray.	Patient died in 1919, cause unknown.

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A207688 9/11/17	M, 33	Farmer, Saskatchewan.	Pain in lower abdomen since removal of ruptured gangrenous appendix in September, 1916. At laparotomy in November many adhesions and much scar tissue at site of previous operation was found. A persistently draining sinus made another operation necessary in March, 1917. Pain became more severe and in June abscess drained over right sacral region and in August abscess over left sacral region.	Distended abdomen, slight tenderness and induration of right lower abdominal wall with two sinuses to right and left of middle of sacrum discharging yellowish pus. Hemoglobin 40 per cent, leucocytes 4,800.	Abdominal actinomycosis with retroperitoneal abscess.	Four transfusions. Sodium iodide intravenously daily, 50 c.c. 10 per cent solution at first, gradually increased to 200 c.c., and tonics.	Patient continued treatment at home. No later report received.
A210659 10/12/17	F, 53	Housewife, husband a furniture dealer, Iowa.	In 1915, dull pain in right upper quadrant radiating to back with nausea and vomiting of bile. In June, acute generalized abdominal pain localizing in right lower quadrant associated with slightly increased temperature, nausea, and constipation. Mass in right lower quadrant one month later.	Large, irregular, slightly movable, and slightly tender mass in right lower quadrant. Hemoglobin 58 per cent, leucocytes 12,000, roentgenogram of colon revealed filling defect in ileum and cecum.	Actinomycosis of ileum, cecum, appendix, and colon, actinomycetes in tissue (Figs. 1 and 2).	At operation, October 17, 1917, ileocecal coil, appendix, part of hepatic flexure, and 10 cm. ileum removed. (Fig. 3). Radium and roentgen ray, sodium iodide intravenously, and potassium iodide by mouth.	In 1919, patient reported condition good, no symptom of recurrence in abdomen.
A211906 10/25/17	F, 30	Housewife, husband electrical engineer, Iowa.	November, 1916, dull pain on both sides of lower abdomen with slightly increased temperature and metrorrhagia. At operation elsewhere in December for suspected ectopic pregnancy, ovaries, tubes, and appendix found in suppurating mass. They were removed. Discharging sinus remained. July, 1917, large mass of cartilaginous consistency removed from pelvis.	Slightly tender mass in hepatic region, large, hard mass in pelvis. Slightly increased temperature and emaciation, hemoglobin 20 per cent, leucocytes 14,000.	Intestinal actinomycosis with extension to pelvis and liver.	Four transfusions between December 4, 1917, and January 3, 1918. April 9, abscess below right breast drained. Frequent roentgen ray, large doses of potassium iodide by mouth, sodium iodide intravenously, tincture of digitalis, and Blaud's pills.	Patient dismissed after six months to continue treatment at home. Death in 1919, exact cause unknown.

TABULATION OF CASES

DATE CASE,	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A224617 3/11/18	M, 26	Farmer, Saskatchewan.	In February, 1917, abdominal distention with chills, fever, and sweats. In April abscess in left upper abdomen discharged green pus. In May and July two more abscesses with persisting, discharging sinuses. Marked loss of weight.	Fluid in left chest and four discharging sinuses in lower and upper left quadrants of abdomen. Temperature 101, pulse 124, hemoglobin 50 per cent, leucocytes 15,000.	Intestinal actinomycosis with secondary involvement of thorax.	Four aspirations of chest. Massive doses potassium iodide for one month. Treatment carried on at home.	Patient died in 1920.
A235668 6/19/18	F, 43	Housewife, husband farmer, North Dakota.	Dull pain in lower abdomen and mass in right lower quadrant for three months with dysmenorrhea and marked constipation.	Mass palpable below the umbilicus, skin over mass bluish red and indurated. Slight anemia.	Intestinal actinomycosis with involvement of abdominal wall.	Roentgen ray, and potassium iodide by mouth.	Several abscesses on abdominal wall ruptured, patient gained in weight, mass not increased in size.
A278991 7/15/19	M, 42	Farmer, Saskatchewan.	Soreness and dull pain across lower abdomen lasting one to three days, marked constipation. In June, 1919, severe attack of pain made operation necessary. Mass at ileocecal coil and many adhesions found, appendix removed.	Slightly tender, hard mass 8 cm. in diameter in right lower quadrant. Roentgenogram revealed filling defect of ileocecal juncture.	Malignancy of cecum.	At operation, July 25, 1919, large mass found involving ileocecal coil and adjacent parts of ileum, cecum, and ascending and transverse colon, 22.5 cm. resected. (Fig. 4). Six arsphenamine injections and massive doses potassium iodide by mouth.	Patient died February 19, 1920. Partial necropsy by home physician showed slight recurrence at site of operation.
A325922 7/21/20	M, 39	Farmer, Saskatchewan.	From 1913 to 1920, seven attacks severe pain in right lower quadrant lasting three to six days. At operation during last attack, ruptured gangrenous appendix and 10 cm. gangrenous bowel removed. Sinus drained since. Marked constipation and loss of 20 pounds.	Hard, indurated mass in right lower quadrant and three draining sinuses. Hemoglobin 50 per cent, leucocytes 13,800.	Intestinal actinomycosis with sinus formation, actinomycetes in pus from sinuses.	Roentgen ray, and massive doses of potassium iodide by mouth.	Patient died January, 1921, from cachexia and extension to thorax.

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A332130 8/31/20	M, 36	Farmer, Iowa.	August, 1919, small lump in right lower abdomen; in March, 1920, it extended to umbilicus. Pathologic diagnosis from piece of excised tissue was chronic inflammatory tissue. Small abscess opened and drained, discharging sinus remained. Two attacks of diarrhea, usually constipation. Eleven injections of autogenous vaccine given without benefit.	Large, hard, irregular mass in right lower quadrant with draining sinus, skin bluish red and indurated. Roentgenogram revealed filling defect in cecum and ascending colon. Hemoglobin 55 per cent, leucocytes 9,800.	Actinomycosis of cecum and ileum involving abdominal wall.	Roentgen ray, massive doses potassium iodide, and tonics.	Patient not traced.
A336397 10/5/20	M, 27	Tile mill owner, Indiana.	May, 1920, loss of weight and strength associated with malaise and cough. In June, attack of pain in right lower quadrant with vomiting lasting one week, small lump in right lumbar region opened and drained, right thigh partially flexed on abdomen.	Large, irregular, hard mass in right lower quadrant with discharging sinus in right lumbar region. Leucocytes 12,000.	Intestinal actinomycosis with sinus formation, pus from sinus showed actinomycetes.	Roentgen ray, and massive doses potassium iodide by mouth.	Patient not traced.
A341474 11/20/20	M, 30	Telegraph operator, Montana.	In 1913, attacks of dull pain across lower abdomen radiating to thigh and right hip, difficulty in extending right thigh. In 1917, severe attack in right lower abdomen, pain radiating to costal margin and right lumbar region. Unsuccessful exploration of gall bladder and right kidney elsewhere followed by profuse discharge pus for seven weeks, mild attacks of pain continued. Operation elsewhere disclosed gangrenous appendix and abscess of abdominal wall, sinus remained.	Four discharging sinuses and hard, tender mass in right lower quadrant, skin over mass bluish red and indurated.	Actinomycosis of cecum and abdominal wall, actinomycetes found in pus from sinuses.	Roentgen ray, radium, and large doses potassium iodide by mouth. Treatment continued by home physician.	Condition much improved after two months.

TABULATION OF CASES

CASE, DATE	SEX, AGE	OCCUPATION, LOCATION	SYMPTOMS AND PREVIOUS TREATMENT	FINDINGS IN EXAMINATION	DIAGNOSIS	TREATMENT	REMARKS
A351274 3/1/21	F, 23	Housewife, husband general merchant.	December, 1918, general abdominal pain localizing in right lower quadrant. Pelvic abscess drained through vagina for three weeks. At exploratory laparotomy elsewhere, appendix, both tubes, and one ovary found in mass of adhesions and removed. January, 1920, recurrence of pain and hard, tender mass in right lower quadrant, second laparotomy. Since then dull pain in abdomen radiating down right hip and side, sinus at old incision drained since June, 1920.	Hard, nodular mass in right iliac fossa and pelvis not connected with uterus, two draining sinuses in lower abdomen, skin bluish red and indurated around sinuses. Moderate anemia, leucocytes 15,300.	Intestinal actinomycosis with extension to pelvis and abdominal wall.	Roentgen ray, potassium iodide by mouth, and sodium iodide intravenously.	Patient still under treatment.

inhabits the gastrointestinal tract, is the cause of the disease. The ileocecal coil is most often affected; the stomach and upper small intestine are practically immune.

5. Carious teeth, diseased tonsils, bad hygienic surroundings, overcrowding, or anything to lessen body resistance are predisposing factors. It is doubtful whether the disease is transmitted from cattle to man. In three of the fourteen cases there was a definite history of exposure to lumpy jaw in cattle.

6. A chronic discharging sinus in the lower abdomen, especially after operation and drainage of an appendiceal abscess, should arouse suspicion of actinomycosis, and repeated examinations for the ray-fungi should be made. A few cases are not diagnosed because the yellow granules are not found at the first examination. Repeated examinations of the purulent material over long periods, with pathologic examination of the tissue from the mass or from the walls of the sinuses, must be made before actinomycosis is ruled out.

7. Early operation and excision of the diseased area before the infection spreads to the surrounding tissues, frequent roentgen ray and radium treatments over the abdomen, and large doses of potassium iodid by mouth and sodium iodid intravenously offer the best hope of cure. In the later stages, after extension of the disease to the adjacent organs and sinus formation, radical surgical measures are of no avail. Some patients apparently are cured under treatment with radium, roentgen ray, and the iodides; others improve wonderfully only to have recurrence in from two to four years.

8. Death usually occurs from cachexia and extension of the disease to the liver and thorax. The average mortality is 60 to 70 per cent.

BIBLIOGRAPHY

[1]Bevan, A. D.: Treatment of Actinomycosis and Blastomycosis with Copper Salts, Jour. Am. Med. Assn., 1905, xlv, 1492-1493.

[2]Chipman, W. W.: The Clinical Aspect of Actinomycosis, Montreal Med. Jour., 1905, xxxiv, 93-98.

[3]Choyce, C. C.: Actinomycosis of the Abdominal Wall, Proc. Royal Soc. Med., 1910-1911, iv, Sec. 79-82.

[4]Cope, V. Z.: A Clinical Study of Actinomycosis with Illustrative Cases, Brit. Jour. Surg., 1915-1916, iii, 55-81.

[5]Dresel, E. G.: Zur Kenntniss der Aktinomykose, Beitr. zur path. Anat. u. z. allg. Path., 1915, lx, 185-226.

[6]Isarel, J.: Seltener Fall von Aktinomykose, Deutsch. med. Wchnschr., 1913, xxxix, 2378.

[7]Keefe, J. W.: Intestinal Actinomycosis, Am. Jour. Obst., lxxix, 13-18.

[8]McCalla, A. J.: Actinomycotic Infection with Case Reports. Can. Med. Assn. Jour., 1919, ix, 411-420.

[9]Ochsner, A. J.: Actinomycosis of the Colon; Ileosigmoidostomy; Treatment of Actinomycosis, Surg. Clin. of Chicago, 1917, i, 1203-1211.

[10]Ramstad, N. O.: Actinomycosis, Jour. Lancet, 1916, xxxvi, 732-735.

[11]Ruhräh, J.: Actinomycosis in Man, with Special Reference to the Cases Which Have Been Observed in America. Ann. Surg., 1900, xxxi, 235-241.

[12]Van Baracz, R.: Report of Sixty Cases of Actinomycosis, Ann. Surg., 1903, xxxvii, 336-340, 463-468.

[13]Wright, J. H.: Biology of the Micreörganism of Actinomycosis, Jour. Med. Res., 1904-1905, xiii, 349-404.

INTESTINAL ANTISEPSIS. EFFECT OF ANTISEPTICS ON A TYPE OF EXPERIMENTAL INTESTINAL TOXEMIA*

By Lester R. Dragstedt, Ph.D., M.D.; Carl A. Dragstedt, M.D.; and Oliver M. Nisbet, M.D., Chicago, Ill.

IT HAS been demonstrated[1] that the poisons, whose absorption is responsible for the toxemia following acute intestinal obstruction, are produced chiefly by the activity of the proteolytic group of intestinal bacteria. It is quite probable that various other disorders may have a similar origin and it is therefore of interest to determine the factors which may influence or control the growth of these organisms. The work of Kendall,[2] Hull and Rettger,[3] Torrey,[4] Cannon,[5] and others has shown conclusively that alterations in the diet may effect striking changes in the character of the intestinal flora. A high protein diet brings about an intestinal flora dominated by proteolytic bacteria whereas a carbohydrate diet, more particularly one containing definite amounts of either lactose or dextrine, suppresses the proteolytic group and brings about a predominance of fermentative or aciduric organisms. Experiments by the authors[6] have indicated that the toxemia incident to intestinal obstruction may be prevented or delayed according to the degree to which the offending proteolytic bacteria can be suppressed by dietary control. Unfortunately for the application of this method in therapeutics it was found that a stasis in the passage of the intestinal content ultimately led to the development of a proteolytic flora and resulting toxemia in spite of the carbohydrate diet.

One of the earliest methods used to check the growth of intestinal bacteria was the administration of the so-called intestinal antiseptics. Notwithstanding many conflicting and contradictory reports it is perhaps the general opinion among bacteriologists at the present time that the administration of these drugs in the methods in current use has little or no effect on the number of viable bacteria which may be found in the feces by cultural methods or the total amount of bacteria as determined by the method of Strasberger.[7] The treatment of various disorders by intestinal antiseptics administered in salol or keratin coated capsules, with the aid of the stomach or duodenal tube with lavage, colonic flushings, or appendicostomy with lavage using antiseptic solutions, is in common use. In general the results have been disappointing except in some specific infections where the chemical used has been specific for the etiologic organism; i.e., emetin in amebic dysentery. Such failures have contributed largely to the widespread opinion that intoxication arising through the activity of the intestinal bacteria plays little or no rôle in pathology.

*Received for publication, August 14, 1922.

Death from acute intestinal obstruction is due to a toxemia. The toxic materials are produced in the intestinal tract by the action of the normal intestinal bacteria on the intestinal contents.[1] The symptoms can be reproduced in dogs by the production of closed isolated segments of various parts of the small intestine by surgical operations. When this is done a toxemia similar to that in acute obstruction occurs and the source of the toxemia is the closed segment since if this be removed the symptoms disappear. The proteolytic group of intestinal bacteria are the principal agents in the manufacture of the poisons absorbed from such closed loops of intestine since if these are rendered sterile by prolonged drainage into the abdominal cavity no toxemia can occur. The production of these closed segments of the intestine is a readily available method of inducing in the experimental animal a toxemia definitely due to the activity of the intestinal bacteria. Accordingly it affords an opportunity for testing the effect of intestinal antiseptics under conditions which can be definitely controlled and in cases where any effect would be easily manifest. In addition the method permits of the direct application of chemical antiseptics to the intestinal mucosa of such strength as would be impossible to secure by other methods.

EXPERIMENTAL PROCEDURE

Dogs were used for this work and the experiments were planned so as to give information relative to other problems of interest as well. This accounts for the large number of animals used. Isolated segments of the

TABLE I

ANTISEPTIC USED	NO. OF EXPTS.	STERILIZATION SECURED AS DETERMINED BY ABSENCE OF TOXEMIA OR NEGATIVE CULTURES
Ether	25	1 (Animal showed no toxemia and examination later revealed a perforated loop with no peritonitis).
Alcohol 70%	10	No segments sterilized.
Lysol 2%	8	No segments sterilized.
Silver Nitrate 5%	4	No segments sterilized.
Zinc Chloride sat. sol.	4	No segments sterilized.
Phenol 5%	5	No segments sterilized.
Salol sat. sol.	11	1 (Animal showed no toxemia and examination later revealed a perforated loop with no peritonitis).
Thymol 10 per cent in alcohol	4	No segments sterilized.
Camphor solid	4	No segments sterilized.
Menthol solid	4	1 (Animal showed no toxemia and examination later revealed a perforated loop with no peritonitis).
Naphthalein	4	No segments sterilized.
Dakin's Solution	3	No segments sterilized.
Beta Naphthol	2	No segments sterilized.
Thymol 10 per cent in alcohol	4	1 apparently sterilized.
Quinine Sulphate sat. sol.	5	No segments sterilized.
Mercuric Chloride	4	No segments sterilized.

upper jejunum were made by dividing the intestine in two places from eight to twelve inches apart and then reuniting the proximal and distal intestine around the isolated segment. The blood supply to the segment was not inter-

fered with and care was taken not to injure the mesentery in any of the subsequent procedure. In most cases the intestinal segment was first thoroughly washed with water introduced at considerable tension and then with the antiseptic to be tested. The results are shown in Table I. In a majority of cases the toxemia incident to the production of the closed loop of intestine was not delayed by the application of the chemicals and in no case was sterilization secured.

Approximately 90 per cent of the animals died within a week following the operation and at autopsy there was found in a majority of cases a marked distention and perforation of the isolated loop with general peritonitis. In a few instances, however, the loops while markedly distended and discolored were not perforated and there were no evidences of peritoneal infection. The material in each of the loops was very toxic when injected intravenously in other animals and contained great numbers of bacteria. Ten per cent of the animals quickly recovered from the operation and never showed any subsequent toxic symptoms. Some of these were operated on again after several months and the loops examined. In most cases these were intact, slightly distended, and contained a thick, gray, foul-smelling, highly toxic fluid. This was found to contain great numbers of putrefactive or proteolytic organisms. The absence of toxemia in these cases has been shown to be due to the astringent action of the chemicals employed which checks the secretion of intestinal juices and consequent distention of the loop, and is not an indication of bactericidal action. Other astringents with no bactericidal properties such an tannic acid have a similar effect. In three experiments the loops were perforated, draining freely into the abdominal cavity, and there were no evidences of peritoneal infection. Cultures taken were negative. In these cases it was thought that a rapid distention and perforation of the loop must have occurred before there was opportunity for any marked bacterial proliferation. Doubtless the previous washings must have reduced the number of bacteria so that when perforation occurred, the bactericidal abdominal fluids could cope with the remainder and so secure sterilization.

DISCUSSION

The clinical use of antiseptics for their action on the intestinal bacteria is limited by a number of factors which play no part in these experiments. Chemicals must be selected which are little if at all absorbed from the intestine and so might cause systemic poisoning, which have no local irritant effect on the intestinal wall, and which still possess antiseptic properties when mixed with the intestinal content. It seems reasonable to suppose that if sterilization of the intestine by chemical means is a possibility it should have been secured in our experiments. Many of the usual antiseptics of a strength thought to be sufficient to sterilize a surgeon's hands were used without regard for their possible toxicity since they could be immediately removed. Furthermore they were applied directly to the segment of intestine in their full strength undiluted or mixed with the gastric or intestinal content. In spite of these most favorable conditions which could rarely if ever be se-

cured in clinical work, in over a hundred experiments it was not possible for us to secure a sterilization or to prevent this type of intestinal toxemia.

CONCLUSIONS

1. The direct application of antiseptic solutions to short segments of the intestine in animals does not effect sterilization or inhibit the production of intestinal poisons.

BIBLIOGRAPHY

[1]Dragstedt, L. R., Moorhead, J. J., and Burcky, F. W.: Jour. Exper. Med., 1917, xxv, 421; Dragstedt, L. R., Dragstedt, C. A., McClintock, J. T., and Chase, C. S.: Jour. Exper. Med., 1919, xxx, 109.

[2]Kendall, A. I.: Am. Jour. Med. Sc., 1918, clvi, 157; Herter, C. A., and Kendall, A. I.: Jour. Biol., Chem., 1908, vii, 203.

[3]Hull, T. G., and Rettger, L. F.: Jour. Bacteriol., 1917, ii, 47.

[4]Torrey, J. C.: Jour. Med. Research, 1919, xxxix, 415.

[5]Cannon, P. R.: Jour. Infect. Dis., 1920, xxvii, 139; ibid., 1921, xxix, 369.

[6]Cannon, P. R., Dragstedt, L. R., and Dragstedt, C. A.: Jour. Infect. Dis., 1920, xxvii, 139.

[7]Harris, N. M.: Jour. Am. Med. Assn., 1912, lix, 1344.

LABORATORY METHODS

SOME POINTS IN METABOLISM USUALLY NEGLECTED BY THE PHYSICIAN*

By J. F. McClendon, Ph.D., and Kenneth March, B.S.

IN THE examination of the diabetic, since it is not possible to determine the quantity of sugar metabolized from the quantity found in the urine or the blood, the only recourse is the study of the complete metabolism. The calculations involved are complicated and hence not often resorted to by the clinician. The first point in this paper is to reduce the work of calculation to a minimum. It is necessary to determine the oxygen consumed, carbon dioxide eliminated, and urinary nitrogen secreted for a given period of time. Owing to the incomplete emptying of the bladder, very short periods may not be used except by catheterization. Folin has shown that the shortest period depending on voluntary emptying of the bladder is three hours. The capacity of portable metabolism-apparatus is usually not sufficient for more than about ten minutes' respiration. In order to obtain three hour values, more than one metabolism period during the three hours is necessary. After determining the urinary nitrogen (by Kjeldahl) calculate the protein-gaseous-metabolism, that is to say, the amount of oxygen and CO_2 concerned in the burning of the protein. One gram of urinary nitrogen corresponds to 6.04 liters of oxygen and 4.88 liters of carbon dioxide. Estimate the total gas metabolism for the whole period of urine collection and subtract from it the protein-gas-metabolism. The result is the nonprotein-gas-metabolism. From the values obtained for carbon dioxide and oxygen, calculate the nonprotein respiratory quotient by dividing the number of liters of carbon dioxide by the number of liters of oxygen. Find this value for the nonprotein respiratory quotient in the first column of the following table. In the second column is given the number of grams of carbohydrate for each liter of nonprotein oxygen. Simply multiply the number of liters of nonprotein oxygen by this figure and you obtain the number of grams of carbohydrate burned during this period. This value is independent of the origin of the carbohydrate whether from glycogen or from the carbohydrate of the food or from the body or food proteins. It is, therefore, the true index of the severity of diabetes. No diabetic is alive who cannot burn some carbohydrate but the less they can burn the more severe is the disease. The number of grams of fat may be obtained by multiplying the figure in the third column by the nonprotein oxygen in liters. This is true at any rate if

*From the Laboratory of Physiological Chemistry, University of Minnesota Medical School.
Received for publication, July 23, 1922.

the fat is totally burned. In case of ketonuria more complicated calculations must be made. The correctness of these calculations is reduced by ketonuria and by the storage of body fat but the storage of body fat rarely takes place in the diabetic and can be temporarily stopped in any person by going on a lower diet, and ketonuria may be reduced to a small value by diet in order to make the determinations, and hence the error will be small. We then have a value for the protein, carbohydrate and fat that is actually burned and if desired may calculate the calories of heat-metabolism by multiplying the number of grams of protein and carbohydrate by 4.1 and fat by 9.3. In fact the heat would be a little greater than this owing to the fact that these figures were obtained in experiments in which there was some loss of food in the feces.

NON-PROTEIN METABOLISM

ONE LITER OF OXYGEN IS EQUIVALENT TO

NON-PROT.	GRAMS	
RESPIRATORY QUOTIENT	CARBOHYDRATE	FAT
.707	0.000	0.502
.71	0.016	0.497
.72	0.055	0.482
.73	0.094	0.465
.74	0.134	0.450
.75	0.173	0.433
.76	0.213	0.417
.77	0.254	0.400
.78	0.294	0.384
.79	0.334	0.368
.80	0.375	0.350
.81	0.415	0.334
.82	0.456	0.317
.83	0.498	0.301
.84	0.539	0.284
.85	0.580	0.267
.86	0.622	0.249
.87	0.666	0.232
.88	0.708	0.215
.89	0.741	0.197
.90	0.793	0.180
.91	0.836	0.162
.92	0.878	0.145
.93	0.922	0.127
.94	0.966	0.109
.95	1.010	0.091
.96	1.053	0.073
.97	1.098	0.055
.98	1.142	0.036
.99	1.185	0.018
1.00	1.232	0.000

If it is merely desired to determine CO_2, complicated apparatus is not necessary. The chief piece of apparatus is one for the collection of the gas sample. A spirometer of fifty liters' capacity is sometimes sufficient for ten minutes' respiration. Valves are used so that the expired air goes into the spirometer. It is very desirable to have the tube entering the spirometer pass through a condenser so as to reduce the temperature of the air to room temperature. For finer work some method of balancing the dome of the spirometer is

desirable. The simplest way to do this is to have a chain from the top of the dome pass up over the pulley wheel, and have a weight attached to the end of the chain equal to the weight of the dome in water, and one centimeter of the chain equal to the water displaced by one centimeter length of the wall of the dome. After you have the air sample in the spirometer, attach a glass tube on the mouth piece, insert this into a two thousand c.c. volumetric flask and by pressing on the dome force five liters of air through the flask and quickly remove the tube and insert the stopper; run in one-hundred c.c. of 0.1 normal barium hydroxide solution containing barium chloride from an automatic pipet and quickly insert the stopper, shaking hard until all of the carbon dioxide has been absorbed by the barium hydroxide; add a few drops of phenolphthalein and titrate back to colorless with 0.1 normal hydrochloric acid; subtract the number of c.c. of hydrochloric acid from one-hundred and multiply the result by 0.56 and the product will be the number of c.c. of CO_2 per liter, of the spirometer air; multiply this figure by the number of liters of air collected in the spirometer and you will obtain the number of c.c. of CO_2 exhaled during this metabolism period.

HEMATOMETRIC DIFFERENTIAL COUNTING*

By A. J. Hinkelman, Oklahoma City, Okla.

THAT in the variations of the absolute number, and consequent numerical relationship of the different kinds of leucocytes found in the blood, in various diseases and pathologic processes, is to be found some specific point of diagnosis, as well as a true index to the body resistance, is something upon which a great amount of work has been spent by clinicians and hematologists of the past, in the effort to establish a definite and practical basis.

However, owing to the lack of a ready means of determining, upon a basis of accuracy, what the differential count really is under various conditions, little success so far has been attained in this direction. The differential count from stained films has long since proved a failure, in a practical way, in obtaining accurate and dependable data upon the subject.

(1) The size and construction of the different cells we have to deal with, vary in such a manner that it is impossible, by any ordinary means, to obtain a uniform distribution upon a slide or cover glass.

No matter how much care is exercised in spreading a drop of blood, or what method is employed, the tendency of the cells will invariably be to arrange themselves into three distinctly different formations, resulting to a greater or less extent, in the possibility of getting three distinct differential counts on the same slide.

The large lymphocytes will always be found in greater numbers along the edges of the slide, than in the middle; while at the beginning of the film,

*Received for publication, July 31, 1922.

or at the end where the drop of blood was deposited, the small lymphocytes will be found proportionally high. Farther on toward the terminal end of slide, the polymorphonuclear cells and the eosinophiles will be found in greater numbers.

(2) Besides the difficulty encountered in this way, there is a process of agglutination of the cells of the blood, with their respective kind, which begins with the process of coagulation and interferes with uniformity of distribution. This is so marked in some cases that even in the short period of time it takes to transfer a drop to a slide and to spread it, there will be considerable grouping of lymphocytes and granular cells respectively.

This difficulty of obtaining uniformity of distribution, in making slide or cover glass smears, has been a matter of much concern among hematologists in work necessitating absolute accuracy of the differential count; so much so that mechanical devices have been perfected in the effort to overcome the difficulty.[1]

However, the difficulty of obtaining uniformity of distribution is not the only obstacle we have in the way of accuracy of the differential count by the slide or dry film method. Even if the uniformity of distribution desired could readily be obtained, the natural distance apart of such cells, normally present only in small numbers, is such that accuracy of enumeration cannot be expected upon a basis of anything less than an impractical number in routine work. A 2 per cent eosinophilia based upon a count of from 100 to 500 cells may in reality prove 0.5 per cent when from 3000 to 8000 cells are counted. Likewise, what appears to be 95 per cent polymorphonuclear cells, based upon the same small number counted, may really only mean 90 per cent or less when calculated upon a fair basis.

Recognizing the fact that the counting chamber is the only logical means of obtaining accuracy of the differential count, many attempts have been made by our earlier workers to perfect fluids that could be used, both as a diluting fluid and a differential stain at the same time. Turk in 1902[2] recommended that 2 to 3 parts of 1 per cent aqueous solution of gentian violet be added to a 1 per cent solution of acetic acid in order to get differential results. This solution, however, does not differentiate the eosinophiles from the neutrophiles and consequently does not answer the purpose in full.

Zappert in 1892[2] found that by filling the mixing pipette to mark 1 with blood and then with a 1 per cent solution of osmic acid up to about half the capacity of the mixing chamber, allowing this to act on the cells for a minute or so, and then filling the pipette to mark 100 with the following solution, the eosinophiles became deeply stained and could be readily enumerated among the other leucocytes.

Distilled water	55 c.c.
Glycerine	45 c.c.
1 per cent Aqueous Eosin Sol.	17 c.c.

The use of this fluid separately, in connection with that of Turk, enabled one to make a complete differential count on the counting chamber. How-

ever, the process is so complicated that apparently it never has gained favor with recent hematologists.

Following the work of Turk, Zollikofer, eight years later,[4] reported a differential fluid that was simpler and enabled one to make a differential count on the counting chamber in a single process. Two solutions are used of the following composition and mixed just before using in equal parts.

A

Yellow Eosin, soluble in water	.05 gram
Formalin, concentrated solution	1 c.c.
Distilled water	100 c.c.

B

Methylene blue "B X"	.05 gram
Formalin, concentrated solution	1 c.c.
Distilled water	100 c.c.

The writer, however, has not found these solutions satisfactory. The differentiation is not clear and the laking of the red cells incomplete.

Unof[5] recommends a mixture composed of equal parts of 5 per cent of formaldehyde, 1 per cent sodium chloride, a 5 per cent aqueous solution of eosin, "W G" and Grubler's polychrome methylene blue solution which is allowed to stain a half hour. This long process of staining in itself, however, would make the process impractical in busy routine work.

In 1919, the writer found that by modifying part A of Zollikofer's fluid by making it 0.5 per cent phenol and 0.5 per cent formalin instead of 1 per cent, this part alone became a perfect and satisfactory combined diluting fluid and differential stain. The formula complete may be set as follows:

Yellow eosin, soluble in water	0.5 gram
Formalin, concentrated	0.5 c.c.
Phenol 95 per cent	0.5 c.c.
Distilled water	100 c.c.

This fluid is staple and may be kept in large quantities in a stock bottle indefinitely. It causes a complete hemolysis of the red cells, swells the white cells to an extent considerably beyond their natural size and stains with the following points of differentiation.

Polymorphonuclear Cells.—These cells take on a brick-dust-red appearance of the granules with the nuclei remaining white and unstained. The tendency of the protoplasm of the cells is to shrink around the nuclei, leaving the cells shaped somewhat according to the number of nuclei present.

Eosinophiles.—Here the granules as well as the nucleus take on a bright red appearance, which may readily be distinguished from that of the neutrophiles, even with the low power. The cells also remain spherical and stand out more prominently than the other granular cells.

Lymphocytes.—The lymphocytes, both small and large, remain hyalin and are distinguished simply according to their size.

The fluid gives a remarkably uniform distribution of the cells on the

counting chamber, apparently due to the action of formalin in preventing coagulation and agglutination to any degree, and enables one to get at once an accurate idea of what the differential count is in cmm. quantities.

Differential Ruling.—The writer has had a special differential counting chamber constructed, which, in addition to the regular Zappert-Ewing ruling, has a marginal ruling of 72 1/10 cubic millimeter fields, or a total ruling of 81 fields. A glance over these fields with the low power enables one to determine the absolute number of the various cells represented, upon a basis of from 3000 to 8000 cells in less time and with less trouble than it takes to make a count upon a basis of 100 cells from a dry film.

Results.—In the regular routine work, of both the laboratory and among hospital patients, the writer has found this method simple and dependable under all circumstances usually encountered. In contrast to the regular method of differential counting, it shows that the differential count is quite constantly a definite character in various infections and pathological processes and a greater classification of diseases and infections according to the character of the blood picture is possible. While presenting a little difficulty, in the beginning, to the technician accustomed to the dry film method, to the beginner, this difficulty is invariably found more easily mastered than the process of proper preparing and staining of blood films. It is only a matter of a little application to become accustomed to readily distinguish the various kinds of cells on the counting chamber, and, after this, accuracy of the count can always be depended upon.

BIBLIOGRAPHY

[1]Hoobler, H. Rexford: Jour. Am. Med. Assn., Feb. 11, 1922, lxxviii, 423.
[2]Wein. klin. Wchnschr., 1902, p. 715.
[3]Centralbl. f. klin. Med., 1892, p. 715.
[4]Ztschr. f. wissen Mik., xvii, 1900.
[5]Woods: Chemical and Microscopical Diagnosis, p. 47.

THE QUANTITATIVE FLOCCULATION TEST FOR SYPHILIS. A COMPARISON OF 500 CASES WITH THE WASSERMANN, USING A SIMPLIFIED SACHS-GEORGI TECHNIC*

By Henry M. Feinblatt, M.D., Brooklyn, N. Y.

THE realization of the nonspecific nature of the Wassermann reaction suggested to many investigators the possibility of devising a simpler test for the serum diagnosis of syphilis. As the belief grew that the Bordet-Gengou phenomenon is largely attributable to the presence of a specific globulin, many workers attempted to invent less involved methods to discover the existence of that substance in the serum.

EARLIER WORK

The conception of precipitating the specific globulin was suggested to Sachs and Georgi by the researches of earlier workers. Michaelis had previously observed that syphilitic sera would sometimes form a precipitate with certain tissue extracts. Porges[1] in 1914 employed a precipitation method; but, when the menstruum was poor in sodium chloride, he obtained false positives. The latter results can be ascribed to the action of too weak a salt solution upon serum globulin.

Subsequently Meinicke used a two phase test. In the first step all of the globulin was precipitated by the addition of water. The second step consisted of the addition of a salt solution, which redissolved serum globulin but had no effect upon the luetic element. Parallel examinations with the Wassermann proved this method to be unreliable.

THE WORK OF SACHS AND GEORGI

Working in Wassermann's laboratory, Sachs and Georgi,[2] in August of 1918, published the results of their researches along these lines. As this article was a war number, it was difficult to obtain, and it was only through the courtesy of the Surgeon General of the Army that the author was enabled to have access to it. Sachs and Georgi described their direct method, which, using only a cholesterinized antigen, produced a flocculant precipitate in syphilitic sera. No hemolytic system was required, the reaction being merely a globulin, antigen, amboceptor combination. Quantitative readings were made according to the degree of flocculation, which was accurately measured by means of the agglutinoscope.

Comparative tests with the Wassermann in 2770 sera yielded complete agreement in 2630 instances, or 94.94 per cent. In 2016 cases both were nega-

*From the Clinical Laboratory, Department of Internal Medicine, Long Island College Hospital, Brooklyn, N. Y.

Received for publication, August 10, 1922.

tive; in 614, both were positive. In the 140 incompatible cases, the Sachs-Georgi was stronger or positive alone in 3.18 per cent; the Wassermann, in 1.88 per cent.

CONFIRMATORY REPORTS

Since that time numerous workers have investigated series parallel with the Wassermann. The results in the main indicate that there is better than a 90 per cent agreement between the two tests; that the Sachs-Georgi is more delicate and appears earlier after the primary sore; and finally that the Sachs-Georgi is a valuable adjunct to the Wassermann.

In October of 1918 Nathan[3] reported his findings in parallel tests with 704 specimens. There was accord in 92.8 per cent. The Wassermann was positive with a negative Sachs-Georgi in 29 cases; in 22 the Sachs-Georgi alone was positive. Nineteen patients from this last group gave a history of preceding syphilitic infection and one additional case was clinically congenitally luetic.

Raabe[4] found a 90 per cent exact agreement. Merzweiler[5] after making 700 comparative examinations concluded that the Sachs-Georgi is a complete substitute for the Wassermann. On the basis of his 1122 tests Messerschmidt[6] reached the same conclusion. Bok employed an improved quantitative technic and believed the flocculation method to be very reliable and more sensitive than the Wassermann. Baumgärtel[7] collected twenty-five thousand cases including seven thousand of his own and found agreement in over 90 per cent.

In this country, Hull and Faught[8] found accord in 88 per cent; Parker and Haigh,[9] in 93 per cent of their 520 cases. Levinson and Petersen[10] obtained parallel findings in 92 per cent of their 1042 tests. Of 62 of their cases in which the Sachs-Georgi was positive and the Wassermann negative, 57, or 92 per cent, gave evidence of lues either in the clinical history or the examination.

In 1921 Harryman[11] performed a parallel series upon 379 fluids from known syphilitic individuals. There was agreement in 91 per cent. In only 5 per cent of the fluids were the findings diametrically opposed. Of these 19 luetic patients, 16 were positive to the Sachs-Georgi alone and 3 to the Wassermann alone. He concluded that the flocculation test is the more sensitive and that it is positive earlier in neurosyphilis.

RELIABILITY OF THE WASSERMANN

It is well recognized that Wassermann reports from different laboratories are frequently contradictory. This is more often due to difference in technic than to error. Solomon[12] reported 3000 cases in which serum from the same specimen was independently examined by two standard laboratories. In this series, which he rightly considers a high water mark of agreement, there were 4 per cent of contrary reports. Considered on this basis, the accord between the Sachs-Georgi and the Wassermann is but slightly less than that which the latter itself gives, when separately performed by two different men.

Of the reliability of the Wassermann reaction where active syphilitic

lesions are present there can be no doubt. White, McWhirter, and Barber[12] report 100 per cent of positive Wassermanns in 177 cases of secondary syphilis, and 98.4 per cent with active tertiary sores. But, where we need it most, in latent lues, congenital syphilis, and neurosyphilis, the Wassermann most often fails us. Any test which can detect a definite percentage of Wassermann-negative cases of this group is entitled to recognition.

The frequency with which latent syphilis and a negative complement-fixation test coexist is suggested by the studies of Symmers, Darlington, and Bittman.[14] These investigators made careful postmortem examinations, both gross and histologic, of 331 cases which had had Wassermann examinations during life. Of those cases which showed definite anatomic evidence of lues, 31 per cent had, during life, been negative to one antigen and 56 per cent to the other.

Serologically negative tabes and meningovascular neurosyphilis are commonplace. The finding of a negative Wassermann with visceral syphilis is so ordinary that resort is often made to the therapeutic test in order to confirm the diagnosis. Consequently, the need is felt of a more delicate test, of one which will pick up certain of the cases which the Wassermann misses.

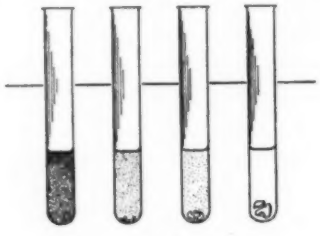


Fig. 1.

TECHNIC

The author's technic differs from the methods more commonly employed in two important respects; i.e., the preparation of the antigen and the use of centrifugalization.

Antigens differ greatly in their sensitiveness, depending upon factors which are not well understood. Individual variations in the tissue extracts selected are probably responsible for much of this lack of uniformity. It was found after repeated trials that supersaturation with cholesterin produces the most sensitive antigens.

After the addition of the antigen to the serum it is common practice to allow the mixture to stand for twenty-four or even forty-eight hours and then make the reading. Hull and Faught[8] first suggested centrifugalization. Gaehtgens in 1906 had used the centrifuge in the performance of the Gruber-Widal reaction, and it appeared logical to employ the same device with the Sachs-Georgi test. Comparative examinations showed that, in addition to being more rapid, the readings are more clean-cut and the flocculation, when present, is more pronounced.

Preparation of the Antigen.—Clean the fat from a fresh bullock's heart, grind the flesh to a fine consistency, and dry with filter paper. Pulverize

the meat with clean sand, simultaneously adding absolute alcohol in the ratio of ten c.c. to every gram of the heart tissue. Place this mixture in glass-stoppered bottles, shake, and incubate at 37° C. for ten days. During this time the mixture should be gently agitated twice daily. Next filter and place the filtrate in stock bottles, to be kept at room temperature.

Cholesterinization of the Antigen.—Add 0.6 gm. of cholesterin per 100 c.c. of antigen to insure supersaturation. Incubate this solution at 37° C. for three or four days, after which it will be ready for use. Prior to the performance of the test, it is preferable to reincubate the cholesterinized antigen at 37° C. for twenty-four hours.

Performance of the Test.—For use in the test the cholesterinized antigen should be diluted in the proportion of one part to nine of 0.9 per cent saline. It is essential to allow no considerable lapse of time to pass between the making of this dilution and the performance of the test. In fact, it is good practice to have the serum already in the tubes so that the antigen may be added immediately after the dilution is made.

Two tubes, each containing 0.3 c.c. of serum, are set up. Two different cholesterinized antigens are employed, one for each tube. To the serum add 1 c.c. of the 1 to 10 cholesterinized antigen, mix, and centrifugalize for fifteen minutes. Upon removing the tubes from the centrifuge, tap each tube gently with the finger and make the reading.

In negative reactions, the solution ascends in a turbid whirl, preserving the original cloudiness of the antigen; but there is no flocculation. In four plus reactions, there is a heavy, flocculant mass, the menstruum being clear. Three, two, and one plus results are represented by lesser degrees of flocculation.

PARALLELISM WITH THE WASSERMANN

Five hundred sera were independently examined by the Wassermann and the Sachs-Georgi methods. The Wassermann tests were performed by Dr. Archibald Murray and Miss Schultz, either in the Hoagland Laboratory or the Polhemus Memorial Clinic, while the author did the flocculation tests in the clinical laboratory of the hospital. The comparative quantitative results obtained in these cases are tabulated in Table I.

TABLE I

PARALLEL OF 500 SACHS-GEORGI AND WASSERMANN TESTS

WASSERMANN		SACHS-GEORGI				
		neg.	one plus	two plus	three plus	four plus
	neg.	*348*	0	4	2	10
	one plus	5	*3*	4	2	3
	two plus	5	0	*2*	1	3
	three plus	0	0	0	*6*	2
	four plus	5	0	5	6	*84*

Explanation of Table I.—This chart is to be read like an annual baseball schedule. The figures placed in the vertical columns relate to the number of sera yielding results to the Sachs-Georgi indicated by the reading at the

head of the corresponding column. The figures placed in the horizontal columns relate to the number of sera yielding results to the Wassermann indicated by the reading at the extreme left of the corresponding column. For example, the figure 10 indicates the number of sera which were four plus to the Sachs-Georgi and negative to the Wassermann; the figure 1 indicates the number of sera which were three plus to the Sachs-Georgi and two plus to the Wassermann. The figures in italics relate to those sera in which identical quantitative results were obtained with both tests.

TABLE II

CLINICAL OPINION IN THE TEN CASES IN WHICH THE SACHS-GEORGI WAS FOUR PLUS AND THE WASSERMANN NEGATIVE

3 cases	congenital syphilis.
2 cases	treated syphilis.
2 cases	interstitial keratitis.
1 case	interstitial keratitis; mother and three sisters have four plus Wassermanns.
1 case	spinal neurosyphilis; mother of luetic children.
1 case	mother of luetic children.

TABLE III

CLINICAL OPINION IN THE FIVE CASES IN WHICH THE WASSERMANN WAS FOUR PLUS AND THE SACHS-GEORGI NEGATIVE

2 cases	congenital syphilis.
2 cases	treated syphilis.
1 case	child with two luetic sisters.

TABLE IV

CLINICAL OPINION IN THE TWO CASES IN WHICH THE SACHS-GEORGI WAS THREE PLUS AND THE WASSERMANN NEGATIVE

1 case	treated syphilis.
1 case	no evidence of lues.

TABLE V

CLINICAL OPINION IN THE FOUR CASES IN WHICH THE SACHS-GEORGI WAS TWO PLUS AND THE WASSERMANN NEGATIVE

3 cases	treated syphilis.
1 case	congenital syphilis.

TABLE VI

CLINICAL OPINION IN THE FIVE CASES IN WHICH THE WASSERMANN WAS TWO PLUS AND THE SACHS-GEORGI NEGATIVE

3 cases	maternity, no evidence of lues.
2 cases	treated syphilis.

TABLE VII

CLINICAL OPINION IN THE FIVE CASES IN WHICH THE WASSERMANN WAS ONE PLUS AND THE SACHS-GEORGI NEGATIVE

4 cases	treated syphilis.
1 case	maternity, no evidence of lues.

An analysis of Table I shows an exact quantitative agreement in 443 sera, or 88.6 per cent. Altogether there were only 31 instances in which one test was positive and the other negative. Thus it may be fairly stated that the two methods were in accord in 93.8 per cent of cases. In 15 sera, or 3 per cent, one test was four plus and the other negative.

ANALYSIS OF DISCREPANCIES

The clinical histories of all patients with contrary serologic findings were carefully examined principally with reference to the presence or absence of other evidences of syphilis. The diagnosis in each case represents the clinical opinion of the attending physician given before he was acquainted with the serologic findings.

TABLE VIII

SUMMARY OF ALL SIXTEEN CASES IN WHICH THE SACHS-GEORGI WAS POSITIVE AND THE WASSERMANN NEGATIVE

6 cases	treated syphilis.
4 cases	congenital syphilis.
3 cases	interstitial keratitis.
1 case	spinal neurosyphilis.
1 case	luetic family.
1 case	no evidence of syphilis.

TABLE IX

SUMMARY OF ALL FIFTEEN CASES IN WHICH THE WASSERMANN WAS POSITIVE AND THE SACHS-GEORGI NEGATIVE

8 cases	treated syphilis.
4 cases	maternity, no evidence of lues.
2 cases	congenital syphilis.
1 case	luetic family.

A consideration of the data presented in the above eight charts offers many points of interest. Fifteen patients with clinical evidence of syphilis, representing 3 per cent of the entire series, reacted positively to the Sachs-Georgi and negatively to the Wassermann. On the other hand, eleven patients with clinical evidence of syphilis, representing 2.2 per cent of the entire series, reacted positively to the Wassermann and negatively to the Sachs-Georgi.

There was only one Sachs-Georgi reading which could possibly be interpreted as a false positive. On the other hand, four maternity patients yielded Wassermann readings which might quite conceivably have been due to the presence in the circulation of placental products. Two of these four cases were followed up after delivery, and in both the Wassermann became negative without antisyphilitic treatment.[15]

Fourteen, or 45 per cent, of the discrepancies occurred in cases of treated syphilis. Here the serum is as apt to be negative to one test as to the other. Accordingly, it would appear advisable to employ both methods in a treated case before reaching a decision as to the need of further antiluetic therapy.

The data here presented would indicate that congenital syphilis, and

interstitial keratitis in particular, is more likely to be positive to the Sachs-Georgi than to the Wassermann.

Finally, it is evident that neither test is infallible and that either one may supplement the other in a definite percentage of cases.

CONCLUSIONS

1. Five hundred parallel Sachs-Georgi and Wassermann examinations showed an agreement of 93.8 per cent.

2. Of the 31 conflicting cases, 16 were positive to the Sachs-Georgi and 15 to the Wassermann.

3. Of the 16 patients who reacted positively to the Sachs-Georgi but negatively to the Wassermann, 15 presented definite clinical evidence of syphilis.

4. Of the 15 patients who reacted positively to the Wassermann but negatively to the Sachs-Georgi, 11 presented definite clinical evidence of syphilis.

REFERENCES

[1]Porges, O.: Die Serodiagnostik der Syphilis mittels der Precipitationsmethoden, Beit. z. Klin. d. Infektionskr., 1914, ii, 419.

[2]Sachs, H., and Georgi, W.: Zur Serodiagnostik der Syphilis mittels Ausflockung durch cholesterinierte Extrakte, Med. Klin., Aug. 18, 1918, xiv, No. 2, p. 805.

[3]Nathan, E.: Zur serologischen Diagnose der Syphilis mittels Ausflockung nach Sachs und Georgi, Med. Klin., Oct. 13, 1918, xiv, No. 2, 1006.

[4] Raabe, Anna: Ueber parallel Versuche mit Serum und Liquor nach Wassermann und Sachs-Georgi, Berl. klin. Wchnschr., Oct. 27, 1919, lvi, 1012.

[5]Merzweiler, K.: Kann die Sachs-Georgi und die Meinicke Reaktion die Wassermann Reaktion in jedem Fall ersetzen? Deutsch. med. Wchnschr., Nov. 13, 1919, xlv, 1273.

[6]Messerschmidt, T.: Vergleichende Untersuchungen zwischen den Reaktionen nach Sachs-Georgi und nach Wassermann, Deutsch. med. Wchnschr., Feb. 5, 1920, xlvi, 150.

[7]Baumgärtel, T.: Wassermansche und Sachs-Georgische Reaktion bei Syphilis, München. med. Wchnschr., Apr. 9, 1920, lxvii, 421.

[8]Hull, T. G., and Faught, Eva E.: Sachs-Georgi Precipitation Test for Syphilis, Jour. Immunol., November 1920, v, 521.

[9]Parker, F., and Haigh, A. V. R.: The Sachs-Georgi Test for Syphilis, Arch. Dermat. and Syph., July, 1921, iv, 67.

[10]Levinson, S. A., and Petersen, W. F.: The Sachs-Georgi Reaction for Syphilis, Arch. Dermat. and Syph., March, 1921, iii, 286.

[11]Harryman, W. W.: The Sachs-Georgi Reaction in the Spinal Fluid of Patients with Syphilis, Arch. Dermat. and Syph., September, 1921, iv, 299.

[12]Solomon, J.: Agreement in Results of the Wassermann Reaction, Jour. Am. Med. Assn., March 20, 1920, lxxiv, 788.

[13]White, C. F., McWhirter, A. T., and Barber, H.: The Wassermann Test: A Criticism of its Reliability, Lancet, London, March 29, 1919, i, 502.

[14]Symmers, D., Darlington, C. G., and Bittman, H.: The Value of the Wassermann Reaction, Jour. Am. Med. Assn., Feb. 2, 1918, lxx, 279.

[15]The author has recently noted weakly positive Sachs-Georgi reactions in two apparently non-syphilitic maternity patients.

The Journal of Laboratory and Clinical Medicine

VOL. VIII ST. LOUIS, DECEMBER, 1922 No. 3

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Chicago

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - DETROIT
FREDERICK P. GAY, M.D. - WASHINGTON, D. C.
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
WARREN T. VAUGHAN, M.D. - RICHMOND, VA.
VICTOR C. MYERS, PH.D. - - NEW YORK
MARSH PITZMAN, M.D. - - - ST. LOUIS

Contents of this Journal Copyright, 1922, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

Modifications of Gram's Stain

IN 1884 Christian Gram observed rather by accident that in kidney sections stained with Ehrlich's anilin gentian violet and Lugol's solution and subsequently decolorized with alcohol, the stain was not removed from certain bacteria. The staining method devised by him at that time, has suffered little modification, and the classification of bacteria as gram-positive and gram-negative has since played an important rôle in bacteriologic studies. During the intervening thirty-eight years the only accepted modifications have been in the nature of rendering the gentian violet solution more stabile. Such an example is Sterling's modification. With a method which has thus stood the test of time, we might well consider variations or attempts at improvement, as entirely superfluous. The relatively rapid deterioration of the stain is however a distinct drawback, and even with the newer modifications the stain is by no means permanent. In many clinical laboratories and even in some bacteriologic laboratories in which the method is used

daily, the stain is allowed to age beyond the period of its reliability. We are inclined to use the same mixture until the bottle is empty rather than until the dye has lost its ability to stain satisfactorily.

It was partly for this reason that the Society of American Bacteriologists has offered standardized methods for the carrying out of the Gram stain. Variations even of these standardized methods have been suggested from time to time and are worthy of consideration and of acceptance if they are found to be of value. Atkins' and Burke's modifications have on the whole been received with approval. Kopeloff and Beerman suggest a further modification in which the advantages of Atkins' and Burke's methods are combined. The method consists briefly in (1) air drying and heat fixation, (2) staining with a mixture of thirty drops of 1 per cent aqueous methyl violet 6 B and eight drops of 5 per cent sodium bicarbonate, (3) fixation with iodin solution containing two grams of iodin, 10 c.c. of normal sodium hydroxide and 90 c.c. of water, (4) draining without blotting, (5) decolorization with 100 per cent acetone, (6) air drying, (7) counterstaining with 0.1 per cent aqueous basic fuchsin.

The authors state that the 1 per cent aqueous violet solution has relatively good keeping qualities and that the bicarbonate neutralizes acidity and improves the intensity of the stain in gram-positive organisms. If these two solutions are mixed, deterioration rapidly occurs, so that they should be kept separately. There is no danger of overstaining. Alkali in the iodin formula is believed to intensify the stain. Acetone apparently is a more satisfactory decolorizing agent than alcohol, and not only is it cheaper, but smaller amounts are necessary.

Kopeloff and Beerman claim for their method better keeping qualities, greater ease of execution and clearer differentiation than by the accepted Gram methods. Detritus which ordinarily takes the positive stain, is stained a pale pink by this method so that this obscuring feature is to some extent eliminated.

Scales has offered another staining method which while differentiating the same group of organisms as Gram's method, does not follow the theory of the method, since together with other differences, the iodin solution is not used. He uses as a stain cotton blue (Poirrier's Blue) made up in 5 per cent phenol solution. Only two solutions are used, the second being a combined decolorizing and counterstaining agent prepared by dissolving saffranin in 95 per cent alcohol and adding an equal volume of acetone. The steps are described as (1) drying and fixation with slight heat, (2) staining with the first solution for twenty or thirty seconds, (3) washing, (4) decolorizing and counterstaining by application of the second solution, (5) washing and drying. Scales reports that in staining sixteen kinds of bacteria his method gave the same positive and negative results as were obtained with the anilin gentian violet stain.

His interpretation of the mechanism of the stain is that a physicochemical reaction occurs. Phenol possesses a great affinity for living organic matter. The dye is soluble in carbolic acid. Thus the phenol serves

to fix the dye firmly within the cell substance. Decolorization and counterstaining appear to be purely physical phenomena in which osmosis through the cell membrane plays an important part. Thus acetone acts purely as a dehydrating agent and if dehydration is carried out rapidly water is completely removed from the cell and the dye remains fixed therein. This also occurs after rapid dehydration with filter paper. On the other hand, if the decolorizing and counterstaining solution contains 25 per cent or less of acetone the decolorization is more complete, showing according to Scales that the dehydration has not been sufficient to reduce the speed of osmosis outward through the cell membrane.

The utilization of acetone in the differential staining of bacteria appears to be a distinct advance. Modifications and improvements of the Gram stain are worthy of consideration and, if found reliable, of acceptance.

—*W. T. V.*

REFERENCES

[1]Kopeloff and Beerman: Jour. Inf. Dis., Nov., 1922, xxxi, 480.
[2]Scales: Jour. Inf. Dis., Nov., 1922, xxxi, 494.

Epidemiology and Bacteriology

THERE seems to be, in the minds of some, confusion as to the relative scope and meaning of the words epidemiology and bacteriology. One man thinks that when he is teaching bacteriology he is instructing his students in epidemiology. This is often far from the truth. Epidemiology is far older and far more inclusive than bacteriology; in fact, compared with epidemiology, bacteriology is limited, and it is one of the handmaids of epidemiology. Two or three hundred years ago when epidemics were more frequent and often, though not always more destructive than at present, there was a word in common use among medical men and the intelligent laity. This word is loimology (loimologia), and was defined as the sum of human knowledge concerning pestilences or epidemics. This word will still be found in our dictionaries among those seldom used, although in common speech and in present-day writings it is never heard nor seen. The dictionaries of today contain another word, lemology, which is supposed to have the same meaning. This word also is obsolete and is not used in spoken or written language. The word epidemiology now bears the same meaning which three hundred years ago was attached to loimology. It means the sum total of human knowledge concerning diseases that prevail or may prevail among the people. Epidemiology, under present or past names, was well established, on at least some scientific basis, long before bacteriology came into existence. The epidemiology of Asiatic cholera was well understood long before Koch discovered the vibrio of this disease. In all human probability, Asiatic cholera has existed in certain parts of India, notably along the Lower Ganges, from time immemorial, but its history begins with the occupation of that country by the English. At that time travel became more extensive, more people

went out from the infected district, and those who did go traveled farther. It was soon recognized that Asiatic cholera is carried only by man, that it goes where the infected man goes, that it stops where he rests, and that there it may again be taken up by some other human being and carried farther. It was demonstrated that Asiatic cholera is not spread by the wind, or carried through the air, or carried by animals, but that its only carrier is man. As the means of transportation increased, Asiatic cholera traveled faster and farther. The first epidemic of which we have a history reached no farther than Asia Minor and northern Africa. Later epidemics took boat on the shores of Asia or from Egypt across the Mediterranean and planted themselves in Europe. Asiatic cholera has traveled by foot, by camel, by ox cart, by sail, by steam, and there is a possibility that it may yet travel by airplane. It was noticed in these epidemics that cholera did not appear in places which protected themselves by quarantine. In the early epidemics it was seen that local epidemics in these traveling pandemics were due to the contamination of drinking water. It was well recognized long before the discovery of the bacillus of this disease that the virus is discharged from the body in the fecal matter. After this discovery it required no great wisdom to recognize the fact that the spread of cholera can be limited by the destruction of the discharges of infected people and by preventing the contamination of drinking water-supplies; in short, the following facts concerning Asiatic cholera were well known and thoroughly recognized before this disease had any bacteriology: (1) It is a specific disease; (2) it never originates *de novo;* (3) its spread depends upon human intercourse between infected and uninfected areas; (4) the direction of its spread is determined by the paths of human travel; (5) places along these lines of travel may protect themselves by quarantine; (6) it is not transmitted through the air by the ordinary mingling of people; (7) it never appears on board ship except when persons infected at the time of sailing are on board; (8) it establishes itself and prolongs its stay in communities the drinking water of which becomes contaminated with fecal matter; (9) even in sparsely settled districts it is introduced and spread by the coming of infected individuals and in this way only; (10) on board ship it is spread from one to the other chiefly through infected water; (11) when a city water-supply becomes infected with the cholera vibrio there is a sudden outburst of the epidemic which soon reaches its maximum and then declines. The decline is due to the killing out of the cholera vibrio by the ordinary bacteria in the water; (12) the cholera virus grows and multiplies in the intestine of man and leads to necrosis of the epithelium; (13) drinking water in any part of the world infected with the cholera virus will cause the disease; (14) while cholera is for the most part distributed by drinking water, there is a possibility of its being distributed, though not widely, by contact, either direct or indirect; (15) cholera may be arrested by quarantine and disinfection. All these facts concerning the epidemiology of Asiatic cholera were known, were recognized, and determined the action of the first International Sanitary Congress held in Constantinople in 1866—long before

Koch discovered the vibrio of this disease; indeed, the discovery of the cholera bacillus has added only one thing to our control of epidemics of this disease. That one thing is an important thing, and it is the recognition of cholera carriers. What is true of Asiatic cholera is along the same line true of many other diseases. When it was ascertained in the fifties of the last century that the typhoid virus is discharged from the one ill with this disease by the bowels and through the urine, a large part of the epidemiology of this disease was explained. It is true that bacteriology has placed in our hands the ability to recognize typhoid carriers and to determine with certainty that transmission by contact may and does occur. When Villemin demonstrated in the sixties of the last century that the sputum and other excretions from tuberculous tissue carry the virus of this disease he became the scientific founder of the epidemiology of tuberculosis. Koch discovered the bacillus, but before him Villemin had demonstrated the contagious and infectious nature of the disease.

The epidemiology of a disease includes much more than its bacteriology, The epidemiologist needs to have some substantial knowledge concerning bacteriology, but the bacteriologist too frequently knows but little of epidemiology. Into the latter there must come, in addition to bacteriology, symptomatology, pathology, avenues of transmission, avenues of infection, habits and customs of the people, knowledge of housing conditions; in short, everything which influences the spread of disease. One would hardly expect to find an epidemic of trichinosis among Mohammedans or orthodox Jews, because neither of these eats pork. Typhus fever is a disease of housing and overcrowding. It has disappeared from certain countries when living conditions have been improved, and this may be *sine qua non* in the eradication of typhus fever. At the present time, with poverty and want so widely prevalent, the immediate eradication of typhus fever from Russia and Poland is nothing more than an idle dream. All claims that this disease has been stamped out by experts sent to countries for that purpose need to be closely investigated before they are accepted. A commission reaches some typhus infested community with the oncoming of hot weather and the disease abates. The coming of the commission and the oncoming of the hot weather fortunately occur simultaneously and the epidemiologist has no hesitancy in determining which of these has been the more important factor in the abatement of the disease. Typhus fever has been almost constantly imported into this country, some years largely, in others sparsely, for a long time and still it has not found a foothold or spread extensively because we are not, on the whole, a vermin-infected people, although we are in spots.

It is idle to talk about general rules of control as applied to all epidemic diseases. Epidemic diseases may be nutritional and wholly independent of any infective agent. They may be bacterial, protozoal, or parasitic. The virus may be air-borne, water-borne, insect-borne, borne by parasites, or disseminated by contact either direct or indirect. It is necessary to study the epidemiology of each disease both apart from and in conjunction with other diseases. Pneumonia as a sequel to measles or influenza is quite another dis-

ease than sporadic primary pneumonia. Much has been learned in the science of epidemiology by observations in armies. For more than one hundred years it has been quite plain to military students of this subject that the mobilization of an army is invariably accompanied by the development of one or more epidemic diseases. This is clearly understood when we recognize the fact that in mobilization a drag net is cast out over the country more or less extensively, as the case may be, and all kinds of infection are brought into camp. One man, possibly from an isolated farm where there was no danger or but little of his infecting others, is suddenly thrust into a great crowd of susceptible men while he bears, without evident harm to himself, a most virulent and deadly infection. French army surgeons recognized this in the epidemics of cerebrospinal meningitis which they studied in the early half of the nineteenth century. The mobilization records of every nation engaged in the late war show similar results. The accession of recruits has often proved more disastrous to an army than a battle. The mortality has been greater; the loss in morale has often been more widespread, and recovery has often been more difficult.

In the epidemics of typhoid fever in our own army during the Spanish War it was repeatedly shown that the incoming of recruits was followed by a recrudescence of the epidemic. Some of the regiments which assembled at Chickamauga during the Spanish-American War went to camp with only eight companies. After typhoid fever appeared in the Park each of these regiments received an accession of four companies. Within from ten to twenty days after the coming of the recruits the epidemic of typhoid fever took on new energy and the number of cases reported daily rapidly increased. Moreover, the new cases were not confined to the recruits, but the number of the old soldiers showing infection also increased. This condition or a similar one was repeated in the observation of the respiratory diseases in every cantonment in the United States during the World War. Typhoid fever and pneumonia epidemics increase not only with the incoming of recruits, but when soldiers from different cantonments mingle. This was so plainly in evidence during the World War that it happened more than once that a relatively small detachment from one cantonment to another changed the dominant type of the pneumonia—causing microorganism in the greater body. There are many points in these well-known observations which might be discussed with profit, but space forbids our doing so at this time.

—*V. C. V.*

Flocculation and Precipitation Reactions in the Diagnosis of Syphilis

THE value of the Wassermann reaction in serologic diagnosis is unquestioned, but the method is not without its real disadvantages. The various ingredients entering into the complement-fixation reaction must be titrated with the utmost precision. Antigens are known to be variable. The German health authorities have attempted to eliminate this uncertainty by furnishing standardized antigen from a central laboratory. Antigen is, however,

but one of several variables. Complement tends to deteriorate with relative rapidity and since the interpretation of the test depends upon the disappearance of complement activity its deterioration may be mistaken for fixation. Again, serum is occasionally either wholly or partially anticomplementary. The former is readily detected with proper controls but in the latter case false positive readings may be made when the serum has absorbed some complement without the addition of antigen. Furthermore, many sera contain a natural hemolysin, the presence of which leads to erroneous negative conclusions. As Keim and Wile point out these and many other technical difficulties undoubtedly explain why, in spite of the fact that the Wassermann reaction has now been in use more than fifteen years, it is not, as yet, wholly dependable. Kolmer and his collaborators have in their extensive researches called attention to the many variables in the Wassermann reaction and have done much toward standardizing the technic.

The fact that alcoholic extracts of nonsyphilitic organs may be used in the test has discredited the specific antibody conception of the reaction. It is chiefly this fact that has inspired recent attempts to simplify the technic. The earlier interpretation of the Wassermann reaction as an antigen-antibody reaction has been questioned and it has been suggested that the phenomena observed are based on colloidal reactions. In 1907 several investigators suggested that a precipitate is formed in the positive Wassermann test and Jacobstahl showed with the ultramicroscope that such a precipitate actually is present when a mixture of Wassermann antigen and syphilitic serum is incubated for one and one-half hours. Attempts to produce a visible precipitation as indicative of syphilitic infection was first successfully carried out by Lange who developed the colloidal gold reaction. Of the ten or more precipitation or flocculation reactions that have been more recently described, the Meinicke, the Sachs-Georgi and the Kahn reactions have, because of their relative simplicity and because of their high correlation with the Wassermann test, received especial attention and study.

It has been shown that the globulins in syphilitic serums and spinal fluids are increased above the normal and the precipitation occurring in the various tests is supposed to be a reaction between seroglobulins and lipoid extract such that the resulting flocculate is a lipoglobulin aggregate. The Sachs-Georgi reaction is one of the simplest so far proposed. The technic consists in the incubation for from eighteen to twenty-four hours of a mixture of inactivated serum, sodium chlorid and cholesterinized heart extract. A positive reaction usually occurs at the end of that time although the tubes are customarily allowed to remain at room temperature overnight, when a second reading is taken. The test has been compared with the Wassermann reaction in over thirty-one thousand cases and in the aggregate there was an agreement of 86.9 per cent in all tests performed by various workers. Levinson states that the test may be elicited earlier and remains positive later than the Wassermann reaction and that it is not too rapidly influenced by treatment. Rice reports more rapid clearing up of the reaction in cases undergoing antisyphilitic treatment. In most of the work reported, the strongly

positive cases and the frankly negative have usually agreed when carried out by both methods. Sometimes, however, positive cases by one method have been questionable by the other, so that the differences have usually been ones of degree only. Rice found a certain number of cases in which there was a total disagreement. That these were in the minority is indicated by his conclusions that when roughly classified into positive and negative, the Sachs-Georgi tests agreed with the Wassermann tests in 99.44 per cent of one thousand cases tested. He found that while the test was less sensitive than the Wassermann in patients receiving treatment and in cases of cerebrospinal syphilis, it was equally as sensitive in untreated cases of primary, secondary and tertiary syphilis. He found in four cases in which the patient's serum was anticomplementary in the Wassermann test, that the Sachs-Georgi test gave a definite and reliable diagnosis in each case. This fact would of itself give the test considerable value in certain cases.

Keim and Wile point out that the main difficulty with both the Meinicke and Sachs-Georgi reactions lies in the fact that the end results are read after forty-eight hours' incubation, at which time the serums frequently show sufficient contamination to lead to false interpretations. The Kahn reaction is read after a much shorter interval and is even further simplified. In it the final precipitate is easily seen with the naked eye and in strongly positive serums spontaneous flocculation occurs, enabling interpretation as soon as the reagents are thoroughly mixed. Serums are mixed with small amounts of diluted cholesterinized antigen and with noncholestrinized antigen and the mixture is then vigorously shaken for three minutes. Sometimes at the end of this period a positive reading may be had, although as a rule readings are made after incubation overnight. The possibility of standardizing this type of reaction is increased by the fact that only one reagent is added to the serum. In primary syphilis there was an agreement between the Wassermann and the Kahn reactions in two-thirds of the cases while the remaining third showed a sensitiveness in favor of the Kahn reaction. Essentially the same findings were made in secondary syphilis. In tertiary lues, the Kahn reaction was found to be not as sensitive as the Wassermann reaction with ice box fixation but more sensitive than the Wassermann reaction performed with the short incubation period. Young in reporting a comparison between the Kahn and Wassermann reactions in five thousand and eighty serums, found an agreement of 93.03 per cent between the two reactions.

Serologic examination for the presence of syphilis has become so generally relied upon that any method which will increase the accuracy of the test should be utilized. Standardization of the original test is of the utmost importance. Other simpler reactions such as the Kahn and Sachs-Georgi tests may be used for confirmatory evidence. It may be that ultimately some simpler flocculation reactions will entirely supplant the Wassermann test. In the meantime, it must be borne in mind that the mechanism of none of the reactions is accurately known. We cannot explain why in syphilis and in practically no other disease some change has been produced in the

blood serum by which the precipitation reactions will become positive. We can explain the phenomenon in terms of colloidal chemistry and effect of proteins thereon, but at present, we cannot demonstrate why such reactions are found in few conditions other than syphilis. Reznikoff, in the November issue of this journal has shown that the colloidal gold reaction is a protein reaction, that the character of the curve varies with different proteins, that the curves are influenced by variations in the albumin-globulin ratio, and that in various clinical conditions such as diabetes, uremia, asthma, hypertension, etc., the amount of protein nitrogen and the percentages of albumin and globulin found in the blood serum vary greatly.

If the gold sol reaction, as a precipitation reaction, precipitates differently in different disease conditions, the possibility of similar variations in the newer flocculation reactions must be borne in mind. On the other hand, the future possibility of differentiation of several diseases by colloidal reactions, is conceivable.

In the meantime, the Kahn test and the Sachs-Georgi reaction appear to have a certain definite value as confirmatory tests when used with the Wassermann reaction. —*W. T. V.*

REFERENCES

Levinson: Amer. Jour. Syphilis, July, 1921, v, 414.
Rice: Jour. Inf. Dis., Nov., 1922, xxxi, 444.
Keim and Wile: Jour. Amer. Med. Assn., Sept. 9, 1922, lxxix, 870.
Young: Jour. Amer. Med. Assn., Nov. 11, 1922, lxxix, 1674.
Reznikoff: Jour. Lab. and Clin. Med., Nov., 1922, viii, 92.

Sprue in Porto Rico

ASHFORD[1] holds that sprue is due to a pathogenic yeast, Monilia psilosis. He believes, however, that this organism can cause the disease only among those living in a state of marked nutritional unbalance. He believes that the agricultural laborers of Porto Rico are living on such diets and he points out what he thinks to be the deficiencies in diet and what the clinical evidences of an unbalanced diet are. (1) A deficiency in fresh animal proteins and in fat-soluble A vitamin; (2) a probable deficiency in certain mineral constituents; (3) a bare sufficiency of B substance, the balance being easily disturbed when fresh vegetable constituents are reduced. These food deficiencies are indicated by the pallor, physical weakness and mental hebetude, together with the thousand and one functional aberrances which are included in the phrase "Effect of the Tropics." There is a very prevalent pigmentation of the skin, especially of the forehead, the jaws, and around the lips. The skin is atrophic and is especially susceptible to mycotic infection, particularly on the legs. Caries of the teeth, with pyorrhea, is highly prevalent. In children there is much nasal catarrh, bronchitis, and disturbances of breathing due to hypertrophied adenoids and tonsils. At all ages there is low blood pressure, with dyspnea, and a tendency to palpitation

[1]Am. Jour. Trop. Med., 1922, ii, 139.

and irregularity of the heart. People frequently complain of chilliness, and on this account they become addicted to the overuse of coffee. (There has never been any great amount of alcoholism in Porto Rico.) Myalgia, neuralgia, and indefinite pains are widely prevalent. Many of the people suffer from mental apathy, have difficulty in concentration of attention, and are defective in memory. In late adolescence there is frequent hystero-epilepsy and spastic conditions are observed in women who become chilled after toasting coffee or in men after unusual exertion. Among women scanty and irregular menstruation is common. Many people suffer from acid dyspepsias, with distention of the abdomen and constipation. There is chronic asthenia and speedy fatigue on effort is almost constantly in evidence. There is a wide prevalence of a waxy sallowness of the face, which deepens into actual and marked anemia, especially when there is infection with hookworm, malaria, or tuberculosis. On people in this condition Monilia psilosis finds suitable soil, grows and multiplies rapidly, and produces the clinical picture which we know as sprue. In a small number of cases sprue occurs in those in which food deficiency plays no evident part and in which the disease seems to come by sheer force of infection, but it is justifiable to suppose that some other factor has diminished the output of the digestive glands so as to permit the successful implantation of the organism.

—*V. C. V.*

Change in Address of the Editor-in-Chief

Until further notice the address of the Editor-in-Chief of this journal will be 535 North Dearborn Street, Chicago, Ill.